NARA | GOAL ONE

August 2011 - March 2013 Cumulative Report

SUSTAINABLE BIOJET

Northwest Advance Renewables Alliance

NARA is led by Washington State University and supported by the Agriculture and Food Research Initiative Competitive Grant no. 2011-68005-30416 from the USDA National Institute of Food and Agriculture.

Goal One: Sustainable Biojet

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

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

The NARA feedstock team is divided into two efforts: feedstock development and feedstock logistics. **Feedstock logistics** efforts have developed models to provide the most cost-efficient collection and transport scenarios of woody biomass. To develop biomass recovery coefficients, a procedure for estimating piled and field distributed biomass has been created and validated. To develop moisture management strategies and models, six tools were evaluated for estimating moisture content in forest biomass, and moisture data from 3000 truckloads carrying forest residuals were analyzed. A communition decision support model for determining the optimal grinding, chipping, and trucking configuration has been completed (Task FL-2; each task progress is detailed in progress reports following this summary). A forest residual sourcing curve showing feedstock costs relative to conversion facility scale was developed for Washington and Oregon (Task FL-1). To evaluate chipping and grinding production to meet alternative feedstock specifications, nine candidate forest harvest residue samples from western Washington and Oregon were identified and collected in approximately 500 oven-dried (OD) quantities. Each sample was screened to remove fines, air-dried, and characterized for polysaccharide, lignin, extractives, ash and bark content. Sample aliquots were distributed to the NARA pretreatment teams for conversion tests (Tasks FL-1 and FL-2). Significant internal outputs to date for this team are listed below. Additional outputs are listed at the end of each progress report.

- Nine residual wood samples representing different species and/or processing have been coded, characterized and distributed to the Pretreatment and Aviation Biofuels teams. Downstream conversion experiments now compare results using the same samples and evaluate conversion robustness on varied residue samples (Figure 2;Task FL-1).
- It was determined that there is very high variability in key quality attributes of the most plausible softwood feedstock for commercial-scale biofuels production from forest harvest residuals. Some of these parameters (bark, species, inorganics, and particle size) can be impacted by harvesting techniques once the value of doing so is communicated to the harvesting operators (Table 1; Task FL-1).
- Feedstock sourcing analysis indicates that forest residuals have the most promising combination of quantity, quality and cost over hog fuel or pulp chips. A sourcing curve was generated for western Washington and Oregon providing a first cut estimate of feedstock costs ($66/BDT delivered) to a 770 thousand BDT/year facility (Figure 4; Task FL-1).
- The decision support model shows that for each 10% increase in grinder/chipper utilization, transport costs are reduced $0.5-$3.0/dry ton. Simulations using this decision support model will be used to inform the Timber Supply Model (Task FL-2).
- Moisture management data has been collected and will be used to predict drying rates for biomass residue piles in western Oregon (Task FL-2).

During the first two-years, the **feedstock development** effort has been refined to focus exclusively on softwood, Douglas fir, to support efforts for production of the forest residuals that are a focus of the project. Commercially viable softwood tree families have been identified. Samples and seedlings are being evaluated for chemical and growth variation. Parameters and protocols used at the WSU Phenomics Center have been established to enable non-invasive high throughput screening of softwood trees to determine their photosynthetic capacity under varied stressed conditions. For these first measurements, trees from the coast and from inland habitats were compared (44 for each habitat) and their response to drought stress analyzed (task FD-2). Fifty-five different families were selected across the range of gains for growth rate. Core samples within these tree families were obtained from a total of 700 trees from three sites. The cores were measured, weighed and shipped for chemical analysis (task FD-3). Ash, carbohydrate, lignin and extractives content were determined for the first 150 samples. In addition, these samples were evaluated for lignocellulosic recalcitrance when subjected to dilute acid pretreatment (task FD-5). To begin linking softwood characteristics to genotype, a preliminary heritability analysis was performed on the first 146 samples (task FD-3). In addition, 15 of the total 83 Forest Service Douglas-fir transcriptome samples have been obtained and analyzed with the intent to identify SNPs (single nucleotide polymorphisms) (task FD-4). Significant internal outputs to date are:

- Variability and probable heritability has been demonstrated in Douglas-fir chemical composition, photosynthetic capacity and lignocellulosic recalcitrance. This information provides a high likelihood that genetic markers can be identified for favorable conversion attributes in softwoods (Tasks FD-2, FD-3, FD-5).

The **Pretreatment Team**'s efforts have progressed with small scale optimization for SPORL, wet-oxidation, dilute acid and mild bisulfite pretreatment options on Douglas-fir wood chips (FS-01) and Douglas-fir residuals (FS-03) followed by mass balance calculations after enzymatic hydrolysis (Tasks C-P-1, C-P-2, C-P-3, C-P-4). The effects of wood chip size on mild bisulfite pretreatment outcomes and bark content on SPORL outcomes were analyzed (Tasks C-P-4, C-P-1). Significant internal outputs to date are:

- Hydrolysate from SPORL and wet-oxidation, and mild bisulfite pretreatment was distributed to and evaluated by Gevo for fermentation analysis (Tasks C-P-1, C-P-2, C-AF-1).

- Solid residue fractions containing lignin from SPORL, wet-oxidation, dilute acid, and mild bisulfite pretreatments were sent to the Co-Products Team for analysis and as reference feedstock material for co-product development.

- A peer-reviewed article was published (Lou et al., ChemSusChem, DOI: 10.1002/cssc.201200859) that demonstrates effective pretreatment at an elevated pH of 5.5 or higher. The lower severity of this process is reduced severity can significantly enhance enzymatic hydrolysis of lignocelluloses, especially substrates produced from the SPORL pretreatment. These findings contradict a well-established and widely accepted concept of “optimal pH of 4.8 – 5.0” that has been exclusively practiced in numerous laboratories throughout the world. By using pH 5.5, we can reduce cellulase loading by approximately 50%. This reduction will have a significant impact on produced fuel costs (Task C-P-1)
- Pretreatment yield efficiencies after hydrolysis provide up to 90% carbohydrate yield. (Tasks C-P-1, C-P-2, C-P-3, C-P-4)

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. Gevo has analyzed pretreated hydrolysate from SPORL, wet-oxidation, and mild bisulfite pretreatments for carbohydrate and inhibitor content. Using SPORL and wet-oxidation hydrolysate from FS-03 residuals, Gevo has determined growth and fermentation performance for a wild-type ethanol producing yeast strain (for benchmark purposes) and for an isobutanol producing yeast strain. They are currently adapting yeast strains for isobutanol production using pretreated hydrolysate from sample FS-03 and have evaluated the performance of advanced strains (Task C-AF-1). Significant external outputs to date are:

- Strain adapted biocatalysts produced a nearly twofold increase in isobutanol over pre-adapted strains on diluted FS-03 pretreated hydrolysate indicating that isobutanol can be generated and that strain adaption can improve biocatalyst performance (Task C-AF-1).

WSU BSEL has optimized fermentation protocols using the BioChemCat process on FS-01 (Douglas-fir woodchips) and FS-03 (Douglas-fir residuals) wet-oxidation hydrolysate to yield volatile fatty acid platform molecules. To extract the platform molecules, a pressurized carbon dioxide extraction system was constructed and 32 trial runs were initiated to optimize the extraction protocols (Task C-AF-2) Significant external outputs to date are:

- Fermentation performance in the BioChemCat process using FS-03 hydrolysate can provide a maximum overall acetic acid yield of 65-70 g/L. The pressurized carbon dioxide extraction protocol has been optimized to remove 85% of acetic acid from water (Task C-AF-2).

Outcomes/Impacts:

Events that cause a change of knowledge, actions or conditions for stakeholders and society.

Training

Name	Affiliation	Role	Contribution
Alvarez Vasco, C	WSU, BSEL	PhD Student	Assay development
Garcia, Karissa B	WSU, BSEL	Undergraduate	Chemical composition analysis
Pedro Guajardo	WSU, BSEL	Undergraduate	High throughput pretreatment
Scott Geleynse	WSU, BSEL	PhD student	Pretreatment and enzyme hydrolysis
Roland Gleisner	USFS, FPL	Tech support	Pretreatment and preprocessing
Shaoyuan Leu	USFS, FPL	Post Doc	Pretreatment of FS-01 and FS-03
Hongming Lou	USFS, FPL	Post Doc	Enzyme and lignin interaction
Chao Zhang	USFS, FPL	Visiting PhD Student	Bench scale pretreatment of FS-01
Haifeng Zhou	USFS, FPL	Visiting PhD Student	Enzyme and lignin interaction
\Rene Zamora	OSU	PhD Student	See Publication List; Task FL-2
Storm Beck	OSU	MS Student	See Publication List; Task FL-2
Fernando Becerra	OSU	MS Student	See Publication List; Task FL-2
Dong-Wook Kim	OSU	MS Student	See Publication List; Task FL-2
Francisca Belart	OSU	PhD Student	See Publication List; Task FL-2
Justin Long	OSU	Res. Associate	See Publication List; Task FL-2
Elvie Brown*	WSU, BSEL	Post Doc	Mass balance of DA and lignin preparation
Ruoshui Ma	WSU BSEL	PhD student	Lignin conversion
Rajib Biswas	WSU, BSEL	Post Doc	100%
Keerthi Srinivas	WSU, BSEL	Post Doc	100%
Nanditha Murali	WSU, BSEL	PhD Student	50%
Ben Garrett	WSU, BSEL	PhD Student	100%

Resource Leveraging

Resource Type	Resource Citation	Amount	Relationship or Importance to NARA
Mond Guo	funded by DOE SBIR		Provided assistance in lignin preparation and enzymatic hydrolysis
Xiaohui Ju	funded by NSF		Provided assistance in lignin preparation and enzymatic hydrolysis
Grant	USDA SBIR Phase II program to Biopulping International (Contract Number: 2010-33610-21589)	$100,000 out of $400,000	Pretreatment of Lodgepole pine and lignin characterization from lodgepole pine
Grant	USDA NIFA-AFRI Competitive Grant (No. 2011-67009-20056)	$100,000 out of 1,000,000	Enzyme and lignocellulose interactions
Scholarship	Chinese Scholarship Council (CSC)	$40,000	Support of Chao Zhang and Haifeng Zhou
Salary	US Forest Service	$45,000/year	Support of J.Y. Zhu & Roland Gleisner
Mond Guo	funded by DOE SBIR		Provided assistance in lignin preparation and enzymatic hydrolysis
Xiaohui Ju	funded by NSF		Provided assistance in lignin preparation and enzymatic hydrolysis
Grant	Collaborating with Humboldt State University on a grant proposal responding to BRDI USDA-NIFA-9008-003828.		Proposal includes testing of alternative grate sizes and alternative screening configurations. USDA rated this as a high priority project, but funding limitations (probably our CAPs) prevented funding. DOE, a co-funder, is now reviewing this proposal.
			The OSU College of Forestry is partnering with the College of Engineering to become a Center of Excellence in Logistics and Distribution as a member of the NSF I/UCRC program. Our participation would be in forest products logistics and distribution and would leverage NARA funding.
DOE		$760,000	Equipment in the pilot facility

FEEDSTOCK

Feedstock Development Team

Task FD-2: Phenomics Analysis

Key Personnel	Affiliation
Helmut Kirchhoff	Washington State University

Task Description

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

The following parameters will be examined because they are expected to be most relevant for growing trees in different designated areas such as: (i) soil composition (N-deprived soil), (ii) fertilizers (iii) salinity, (iv) drought. Softwood trees will be used which passed the first selection criteria for maximal biomass production and quality. We expect to screen about 500 individuals. For each of the four parameters, a set of the 500 selected individuals will be grown and screened in parallel (about 2000 plants total). Beside morphometric parameters (leaf size and number, chlorophyll content), the maximal photochemical quantum efficiency, photosynthetic electron flux, capacity of photoprotective non-photochemical quenching, as well as the degree of photoinhibition of photosystem II will be deduced from chlorophyll fluorescence measurements. This detailed analysis will indicate the stress status of the trees under the different environmental parameters and will allow selection of the most robust plants.

Activities and Results

In order to optimize the equipment and protocols used to perform phenomics experiments on trees, initial parameters were established on well-studied plants in the genus *Arabidopsis* and *Populus. Arabidopsis* mutants with reduced lignin content were studied in the initial work. Low lignin content (higher cellulose/lignin ratio) is a desirable trait for biofuel production from woody biomass. By using mutants affected in lignin biosynthesis, we aim to study the impact of low lignin content on photosynthesis. That knowledge is required for optimizing feedstock since photosynthesis forms the energy source for plant biomass. Growth curves showed that these mutants grew very similar to wild type plants indicating that reduced lignin content has no direct impact on growth. An interesting result is that the light-induced proton motive force (pmf) required for ATP production is higher in the mutants compared to their wild-type counterpart. A higher pmf in the lignin mutant is indicative for a slower consumption of ATP caused by altered lignin metabolism. Furthermore, the photoprotective mechanism (qE parameter = high energy quenching) that is activated in plants to avoid damage by high-light is less in lignin mutants. Overall, this data has given indication for feedback between lignin biosynthesis and photosynthesis. Unraveling this interrelationship can pave the way to optimize trees with high cellulose/lignin content.

Young poplars (height 1m) were used to establish optical measurements in the Phenomics Center because these types of measurements were not reported so far. After establishing the measurements, studies were focused on photosynthetic performance and on the ability to avoid photooxidative stress by activation of photoprotective processes (qE parameter). Comparison of the different poplar hybrids showed significant differences in photosynthetic parameters. The hybrids A19, A44, 1732-48, and P3 have much lower photosynthetic performance values whereas A16, 2R-35, 1732-85, and 1732-84 have higher values compared to the control trees. We could not identify a correlation between the photosynthetic performance parameter and photoprotective qE mechanism. Thus, the differences in photosynthetic performance seen in the different hybrid trees do not seem to be correlated with the ability to activate photoprotection on the level of the photosynthetic apparatus. The data indicates that the different poplar hybrids have distinct photosynthetic features.

In the Phenomics Center, we performed, for the first time, a large-scale phenotyping of Douglas-fir trees. For these first measurements, trees from the coast and from inland habitats were compared (44 for each habitat) and their response to drought stress analyzed. A main outcome of these measurements is a high variability in photosynthetic parameters as shown in Figure 1 for the Fv/Fm parameter. Furthermore, drought stress over eight days did not lead to detectable phenotypic changes (both in RGB images as well all other photosynthetic parameters), indicating a high robustness against drought stress. Finally, both coastal and inland grown plant types show no significant differences. The experiment will be repeated for longer drought period to identify individuals that can withstand drought longer and individuals that are more sensitive. The work was performed in the WSU Phenomics Center (Pullman, WA). Experiments were run by Magnus Wood (not supported by NARA). In November 2012, a new PostDoc (Ricarda Hoehner) was hired for the NARA Phenomics work.

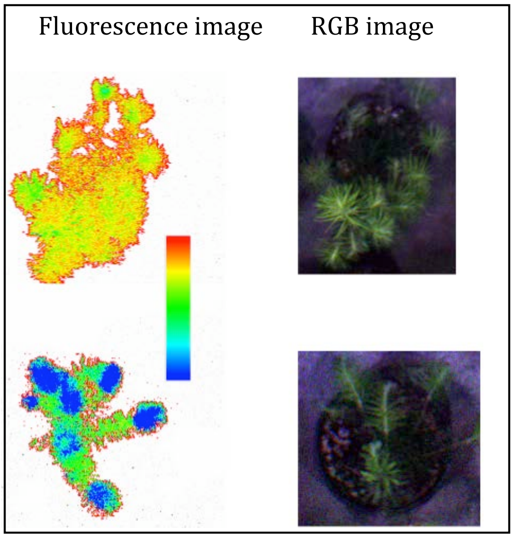


Fig. 1. Fv/Fm parameter on multiple Douglas-fir individuals as a response to drought stress.

Recommendations/Conclusions

Methods to study young trees were established and first results indicate significant differences in photosynthetic performances of poplar hybrids and in Douglas-firs. Since phenomics is a new approach, the first aim was to establish the non-invasive optical screening for the plants. We could solve different practical problems (e.g. mutual obstruction of fast growing plants, parasite infestations). Developing a SNP chip for in-depth genetic analysis on Douglas-fir has high priority. In this respect, the Phenomics approach will act as a gatekeeper that can identify individuals with desired traits that should be selected for more elaborate, expensive, and time-consuming approaches (e.g. chip technologies). For example, determining plant phenotypes under drought stress can detect drought resistant and sensitive individuals. Differential genotypic analysis on these selected plants promises to unravel key genes or gene patterns that can lead to drought resistant trees and guide breeding strategies for feedstock optimization.

Physical and Intellectual Outputs

None to report at this time

Task FD-3: Combining Genomic and Field-based Breeding and Testing Methods to Improve Woody Feedstock Production

Key Personnel	Affiliation
Keith Jayawickrama	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, as well as to reduce cost. Recent advances by the Conifer Translational Genomics Network (a multi-institution project for major US conifers) can be put to use in this project. 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) 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

The progeny test populations most suitable for sampling should: (1) have advanced-generation high-genetic gain germplasm, (2) have trees large enough to obtain amounts of wood needed for chemical analysis, (3) have good maps and access information, and (4) be available and accessible to OSU researchers and contractors. Two second-generation populations, T96 and CL98, established by Plum Creek Timber Company in 1996 and 1999 respectively, were selected. Various sampling tools (cordless drills, gas-powered drill, 5mm, 10mm and 12 mm manual corers) were compared and the 10 and 12mm manual corers with extensions were selected to produce the core samples.

Fifty-five (55) different families were selected across the range of gains for growth rate from the T96 population. Samples within these tree families were obtained from a total of 700 trees from three sites. The cores were measured, weighed and shipped to Xiao Zhang's lab at Washington State University. Enough sample quantity was obtained to provide 10g of dry wood for analysis. The final set of 150 cores were dried and ground in a Wiley mill at OSU to free up time for the Zhang lab to expedite analysis.

Preliminary analyses on the first 146 samples showed very high heritabilities (abbreviated h^2). A heritability of 0 means there is no genetic control, a heritability of 1.0 means variation in the trait is totally genetic. These results strongly indicate that Douglas-fir would respond to genetic selection for these 3 traits.

	Klason_Lignin	Holocellulose	Extractives
Narrow-sense individual h^2	0.823 ± 0.200	0.778 ± 0.1949	0.000 ± 0.000
Broad-sense individual h^2	0.823 ± 0.200	0.778 ± 0.1949	0.650 ± 0.349

We selected 30 more families and 3 woods run lots from the CL98 series, located and visited the Moon Creek progeny test site near Fairview, and collected a first batch of samples.

We started collaborating with Helmut Kirchhoff's lab to pursue phenomics work with Douglas-fir, with emphasis on drought tolerance. Two sets of containerized seedlings were sent to the lab, one from a moister coastal breeding zone and the other from a drier inland southern Oregon breeding zone. The objective of this pilot study is to get baseline parameters for Douglas-fir. Three different parameters (false color Fv/Fm, NPQ, PhiII) were evaluated after 8 days without water. In this preliminary study the two populations did not appear different.

The second phase was to leverage a Douglas-fir Drought-Tolerance study with the USDI BLM, and sow seedlings from 107 elite families (originating from across western Oregon and western Washington) and two woods run lots for detailed analysis (including fluorescence assay) by the Kirchhoff lab. We visited the BLM nursery at the Sprague seed orchard near Pleasant Valley to coordinate the sowing of these drought tolerance study seedlings. The seedlings have germinated and are in the process of being transplanted to the 615A cavities.

Sub-Task 2. Identify single nucleotide polymorphism (SNP) genotypes

James Cook, Callum Bell, and Glenn Howe (OSU faculty member) reviewed and updated plans for genotyping Douglas-fir. James Cook requested and obtained approval from NARA Leadership team and WSU leadership for modification to the budget allocation (emphasizing softwoods instead of poplar).

Gave input to NCGR on obtaining Douglas-fir sequence data from Rich Cronn (USDA PNW Research Station, Corvallis). Continued to update research priorities based on genotyping and genomic selection research published in early 2013. Began simulation work to devise an efficient multi-generation sampling strategy to obtain the maximum information from future sample collection and genotyping work. Opportunities to form a Douglas-fir genotyping consortium are being explored, with the objective of lowering genotyping costs by capturing economies of scale.

Recommendations/Conclusions

As expected, study of chemical composition and degree of biomass recalcitrance at the individual-tree level is time-consuming and not feasible for a large number of programs (although it yields the maximum data from the genetic analysis viewpoint). However, the individual-tree data from T96 is necessary to calculate heritabilities, correlations between traits, and potential genetic gains for these traits. Large variations have been found between trees and families for the traits of interest and it is apparent the traits will respond to genetic selection.

Sample collection from the CL98 series was completed and samples were processed and shipped to WSU/Tri-Cities for wood chemistry analysis by the end of April. Wood samples from future progeny test series will be bulked by family, reducing the number of samples by a factor of 8-10. This will allow us to work through many more programs in years 2-5 than in the first year. We delivered bulked samples from the second population (CL98) by the end of April 2013. However, once we identify the families in each program with the most desirable fuel properties, we would like to have individual samples analyzed within these few top families to find the individuals with the best properties and propagate them by grafting. This will allow for future seed production. This will include more advanced-generation programs for Douglas-fir and also one western hemlock program.

We expect to deliver about 1,000 seedlings from 107 families and two unimproved controls for the drought tolerance study to the Kirchhoff lab by September 2013.

Personnel and resources are in place to move forward rapidly on the SNP genotyping program. We envision developing a sampling strategy with the aid of the simulation mentioned above, and starting tissue collection and DNA extraction before the end of year 2. Inferring from two recent publications on Douglas-fir, we may able to move forward in short order with existing SNP databases to build a chip and then to start genotyping.

Physical and Intellectual Outputs

Research Presentations

Alvarez-Vasco, C.A, X. Zhang, K.J.S. Jayawickrama and K.B. Garcia. 2013. Phenotypic variations of biomass recalcitrance in Douglas fir families. Poster accepted by the 35th Symposium on Biotechnology for Fuels and Chemicals, Portland, OR, April 29-May 2, 2013.

Alvarez-Vasco, C., K. Garcia, K. Jayawickrama, E. Brown and X. Zhang. 2012. High throughput analysis of biomass recalcitrance of Douglas fir families. Panel presentation at NARA 2012 Annual Meeting, Missoula, MT, Sept 13-14, 2012.

Alvarez-Vasco, C., K. Garcia, K. Jayawickrama, E. Brown and X. Zhang. 2012. Variation in Chemical Composition and Biomass Recalcitrance of D. fir Families. Poster presentation at NARA 2012 Annual Meeting, Missoula, MT, Sept 13-14, 2012.

Jayawickrama, K.J.S., T. Ramaraj, and C.J. Bell. 2012. Marker-aided selection for biofuel production potential in Douglas-fir. Poster presentation at NARA 2012 Annual Meeting, Missoula, MT, Sept 13-14, 2012.

Task FD-4: Genetic Variation Underlying Amenability to Pretreatment/Bioconversion

Key Personnel	Affiliation
Callum Bell	National Center for Genome Resources

Task Description

NCGR (National Center for Genomic Resources) will build an updated reference transcriptome for Douglas-fir. We will draw on the two existing references (Howe et al., Wegrzyn et al.) plus newly available single-ended 101 base pair Illumina reads from a collection of 83 trees (US Forest Service). NCGR will apply its Batched Parallel Assembly (BPA) software to these Illumina reads. The workhorse of the assembly is the ABySS assembler, which applies a De Bruijn graph approach. The ABySS kmer pool is fed into an overlap consensus using Mira to further contiguity and collapse redundancy. If the pool is very large (>several million sequences), a cd-hit-est can be performed to reduce the set to the best two cluster representatives. The final synthetic-EST set is then deredundified further using cd-hit-est if desired, and is fed into alignment using BWA. The existing reference transcriptome contigs can be fed into the BPA pipeline at the Mira stage, which will yield a new transcriptome reference that unifies the available resources.

SNP (Single Nucleotide Polymorphism) discovery and prioritization (Task FD-4.4) will be done by alignment of the newly available Illumina sequence reads to the updated reference transcriptome, and identification of mismatches. NCGR will do this by applying Alpheus, its high-throughput alignment and variant detection pipeline. Alpheus is a parallelized workflow that aligns short reads to the reference using the GSNAP algorithm, and parses the output to tabulate all variants that are discovered along with supporting statistics such as the number of reads having a variant, variant nucleotide quality, and sequence coverage at the variant position. Threshold heuristics are then applied to arrive at a draft set of putative variants that have strong support. Alpheus will be supplemented by bioinformatics methods already applied in Douglas-fir genetics (Howe et al.) to arrive at a candidate set of new SNPs ready for validation. The goal is to supplement the existing SNP set with new polymorphic variants, with the particular goal of having SNPs in every Douglas-fir gene.

Activities and Results

An updated transcriptome assembly for Douglas-fir is now available from the USDA Forest Service (Table 1). It was assembled from 36 of the Forest Service transcriptome samples. This reference is stranded and twice the size of the previous two references available.

NCGR has gained access to sequence reads for 15 of the total 83 samples that the Forest Service maintains. We intend to use all 83 samples in our comprehensive transcriptome reference, but a trial assembly was performed to determine whether the efforts would still be valid after the release of the Forest Service's new reference. The first eight samples received were assembled using NCGR's BPA software (Table 1). We expect the size of the assembly to increase as data from additional samples are introduced into the assembly.

The ability to align sequence reads to a reference is critical in SNP identification. This quality was assessed in both the new Forest Service reference and our trial assembly (Table 1). On average, 6% more reads aligned to NCGR's trial reference than to the Forest Service reference. This could amount to a substantial number of reads over the entire set of 83 samples. We still recognize that SNP identification would benefit from an updated, comprehensive transcriptome assembly for Douglas-fir.

Table 1. Summary statistics for current Douglas-fir transcriptome references.

Reference	No. Contigs	Total bases (Mb)	N50 (Kb)	Mean % of reads aligned
FS (short read)	39,000	36.5	1.3	-
JGI (454)	25,000	32.5	1.6	-
FS (w/UC-Davis, stranded) *NEW	90,730	78.1	1.2	78
NCGR (trial, 8 sample)	41,140	38.7	1.8	84

Recommendations/Conclusions

NCGR is ready to start assembling the Douglas-fir transcriptome once data from all 83 samples are made available. When the assembly is complete, we are prepared to identify SNPs using our variant-detection pipeline, Alpheus. Two recent publications make further reevaluation of the project necessary:

Glenn T Howe, Jianbin Yu, Brian Knaus, Richard Cronn, Scott Kolpak, Peter Dolan, W Walter Lorenz and Jeffrey FD Dean. 2013. A SNP resource for Douglas-fir: de novo transcriptome assembly and SNP detection and validation. BMC Genomics 14:137.

Müller T, Ensminger I, Schmid KJ. 2012. A catalogue of putative unique transcripts from Douglas- fir (*Pseudotsuga menziesii*) based on 454 transcriptome sequencing of genetically diverse, drought stressed seedlings. BMC Genomics. 13(1):673.

Between them, these studies may provide sufficient SNPs to create a useful genotyping resource without further bioinformatics work on the Forest Service sequence reads. We are in the process of reevaluating the available resources and formulating a new plan. There may be the opportunity to join an international consortium, which has the potential to reduce costs by gaining access to bulk discounts through Illumina. At the same time we should not ignore new technologies such as genotyping-by-sequencing (GBS).

Physical and Intellectual Outputs

Research Presentations

Jayawickrama, K.J.S., T. Ramaraj, and C.J. Bell. 2012. Marker-aided selection for biofuel production potential in Douglas-fir. Poster presentation at NARA 2012 Annual Meeting, Missoula, MT, Sept 13-14, 2012.

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 major barrier hindering the development of an economically viable biomass conversion process. Softwood is the largest biomass feedstock in the Pacific Northwest. However the biomass recalcitrance variations among different softwood species and families are not known. The first objective of the task is to determine the biomass recalcitrance variations among Douglas-fir families and to screen and identify the "most" and "least" Douglas-fir lines.

The genetic influence on biomass recalcitrance is a poorly defined area. Elucidating potential genetic factors influencing biomass recalcitrance can provide breakthrough understanding to the selection and breeding of "ideal" biomass feedstock for biofuel and bioproducts conversion. Approximately 3,000 Douglas-fir lines of different families will be tested for biomass recalcitrance. The work will generate a significant amount of information and allow detailed genetic analysis to identify potential genetic trait(s) controlling biomass recalcitrance. Our second objective is to provide biomass recalcitrance information from different Douglas-fir families to the genetic analysis group (Keith Jayawickrama, Callum Bell, Helmut Kirchhoff) to elucidate and understand the genetic influence on biomass recalcitrance.

To attain a comprehensive understanding of biomass recalcitrance, we devised a three-step screen protocol (Figure 1). The first step is a high throughput chemical compositional analysis to determine the amount of total carbohydrate and lignin in the different Douglas-fir families. Douglas-fir core samples (approximately 20cm length × 1cm diameter) were taken from breast height of each Douglas-fir tree lines (age 16 yr). The samples were ground and passed through a 1 mm screen on a Wiley mill. Wood particles with sizes between 20-60 mesh were collected for the analysis. We chose to determine klason lignin, holocellulose and acetone extractives to represent the chemical compositions of the samples. The klason lignin was determined following Tappi standard assay (T222) with 100mg wood samples. The holocellulose content was determined by using acid chlorite treatment of wood samples. The amount of holocellulose represents total carbohydrate content. The acetone extractives content was obtained by extraction of wood in acetone solvent for four hours. The acetone extraction, acid chlorite treatment and 3% acid hydrolysis were carried out in an Ankom 200 fiber analyzer incubator where 24 sample analyses can be processed simultaneously.

Biomass recalcitrance can be attributed to both chemical and physical/structural recalcitrance. The ultimate goal of biomass conversion is to attain an efficient and high yield conversion of wood carbohydrate to biofuel. While a feedstock with higher holocellulose content can potentially provide more sugar for fuel production, its physical characteristics, such as density, fiber length and cell wall thickness, also have a significant impact on the susceptibility toward subsequent pretreatments to release the chemical components. The second step of the protocol is the pretreatment screening of Douglas-fir lines. Two pretreatment chemistries were applied, diluted acid (DA) and alkaline hydrogen peroxide (HP) pretreatment. DA is one of the most common biomass deconstruction methods used on all types of biomass. In a typical DA process, biomass hemicelluloses are preferentially hydrolyzed while most of the cellulose and lignin remain in solid substrate. Alkaline hydrogen peroxide pretreatment of Douglas-fir family lines was used to compare between different pretreatment conditions. HP pretreatment is expected to target on lignin degradation to improve substrate digestibility to the enzyme. Pretreatment of different Douglas-fir family lines were carried out in tube reactors, a modified procedure adapted from the National Renewable Energy Laboratory (NREL). Twenty-four samples were pretreated at the same time in the tube reactor placed in an oil bath. Both water soluble fractions (WSF) and solid fractions after the pretreatment were collected. The WSFs were analyzed for releasing sugars and soluble phenolic compounds measured by high throughput microplate assay. These results provided information on recalcitrance of each Douglas-fir family lines toward carbohydrate and lignin removal during pretreatment. The holocellulose and lignin remaining in the solid substrates were also determined. The solid substrates were subjected to enzymatic hydrolysis in the third step. A relatively excessive amount of enzyme (~50mg cellulose per gram of cellulose) was applied to hydrolyze these substrates and glucose released after 72 hours of hydrolysis was determined. High enzyme dosages were applied to minimize the effect of potential enzyme dosage limitation while maximizing the effect of substrate digestibility differences among different Douglas-fir lines.

NREL has developed a high throughput screening method based on suing microtiter plate assisted by robotics to determine the sugar released from combined pretreatment and hydrolysis. A large amount of sample analyses can be performed, however, relatively limited information can be obtained. Our screen method can provide a more comprehensive understanding and quantification of biomass differences among different samples, which are critical to the subsequent genetic analysis to identify potential genetic traits associated with biomass recalcitrance.

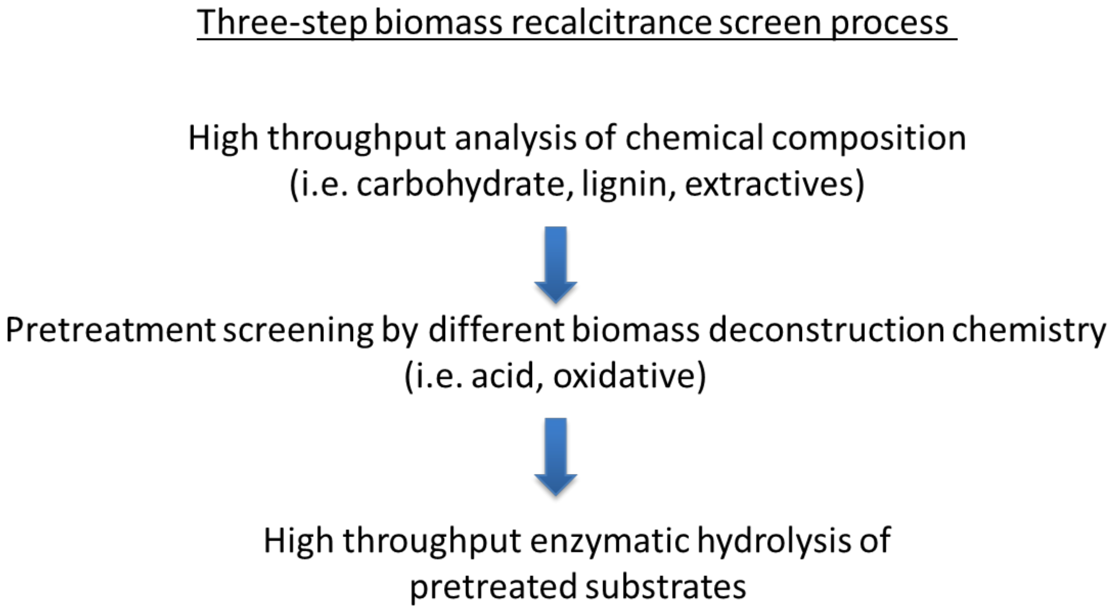


Figure 1: A three-step screen protocol to determine the degree of biomass recalcitrance of softwood

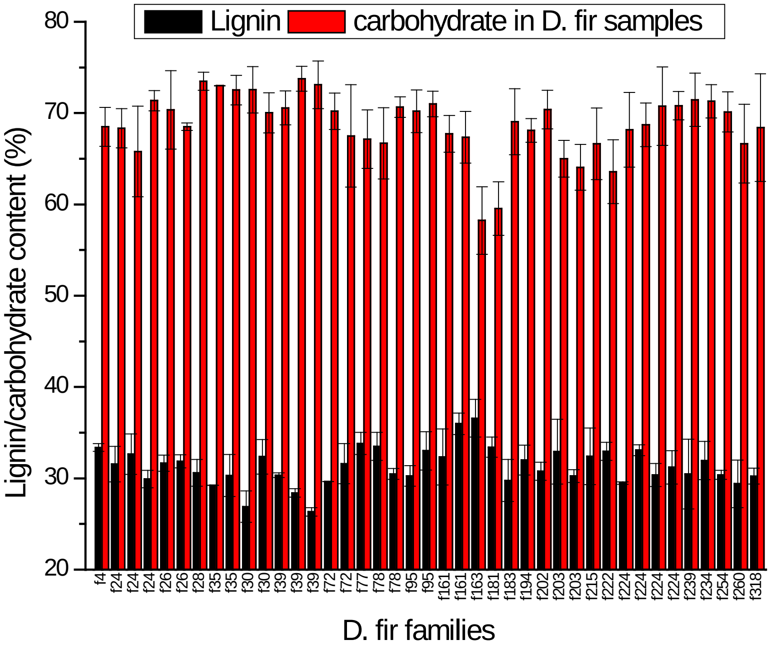
Activities and Results

At the beginning of the project, the work was focused on developing a reliable screen protocol and refining each assay step. This development also included working with Oregon State University to standardize the tree sampling procedure to obtain representative and sufficient samples for analysis. A significant amount of replicated experiments were carried out to verify the results obtained from this screening method and comparing this method with standard or conventional testing protocols for chemical analysis (lignin and sugar analysis), pretreatment and enzymatic hydrolysis.

Since June 2012, ~ 500 core samples from different Douglas-fir family lines (from different families or same families planted in different locations) were received. A detailed analysis of the first batch, 150 samples analysis, was completed. A significant variation in chemical composition was observed in different Douglas-fir family lines. Lignin, holocellulose and extractive amounts varied between 26-38%, 54-76% and 0.6-5% respectively (Figure 2). Dr. Jayawickrama conducted an initial heritability analysis and the results suggest that the variations in lignin and holocellulose content are under strong genetic control. This analysis will be confirmed once we complete the remaining samples from the initial 500 Douglas-fir phenotypes.

To identify suitable pretreatment conditions for Douglas-fir lines, more than 20 different pretreatment conditions from each of DA and HP chemistry were first evaluated on FS01, a reference Douglas-fir sample used by the NARA pretreatment team. Diluted acid pretreatment of Douglas-fir using 4% sulfuric acid (w/w on wood) for 30 minutes at 180°C and alkaline peroxide pretreatment of Douglas-fir at 180°C for 30 minutes using 4% hydrogen peroxide (w/w on wood) were selected. These are the relatively mild pretreatment conditions to produce substrates with moderate substrate digestibility. A significant variation on solid substrate yield from diluted acid pretreatment of different Douglas-fir lines was observed between 58% - 71% (Figure 3). The holocellulose content in pretreated Douglas-fir lines varied from 50-58%. The holocellulose content after HP pretreatment was higher, ranging from 60-70%. The variation in the solid substrate yield difference after HP pretreatment appears to be less significant compared to diluted acid pretreatment. It is interesting to find a huge difference among the digestibility of different Douglas-fir family lines after pretreatment (Figure 4). The conversion yield from diluted acid pretreatment differs from approximately 19% to almost 50%. This variation appeared to be the most significant among other parameters. This suggests that biomass recalcitrance is not determined by a single biomass property. It was also interesting to find that some Douglas-fir lines exhibited low recalcitrance toward both pretreatment methods (e.g. 3996, 4665) while other showed different levels of recalcitrance toward different biomass deconstruction methods. A number of highly recalcitrant and less recalcitrant Douglas-fir lines were identified. We are currently investigating the relationships between the substrate digestibility and other parameters (composition, pretreatment yield, etc.).

A: lignin and carbohydrate



B: Acetone extractives content

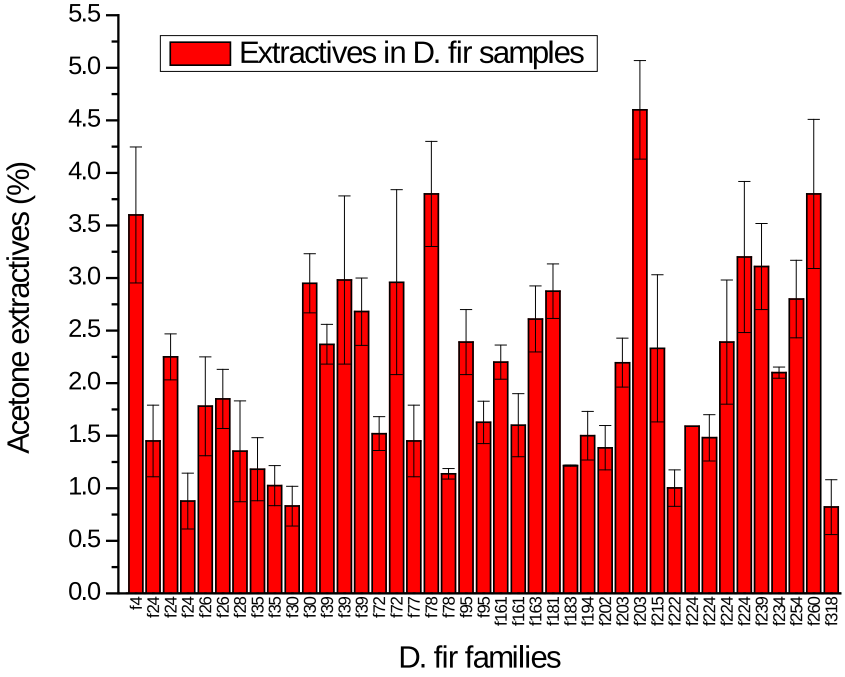
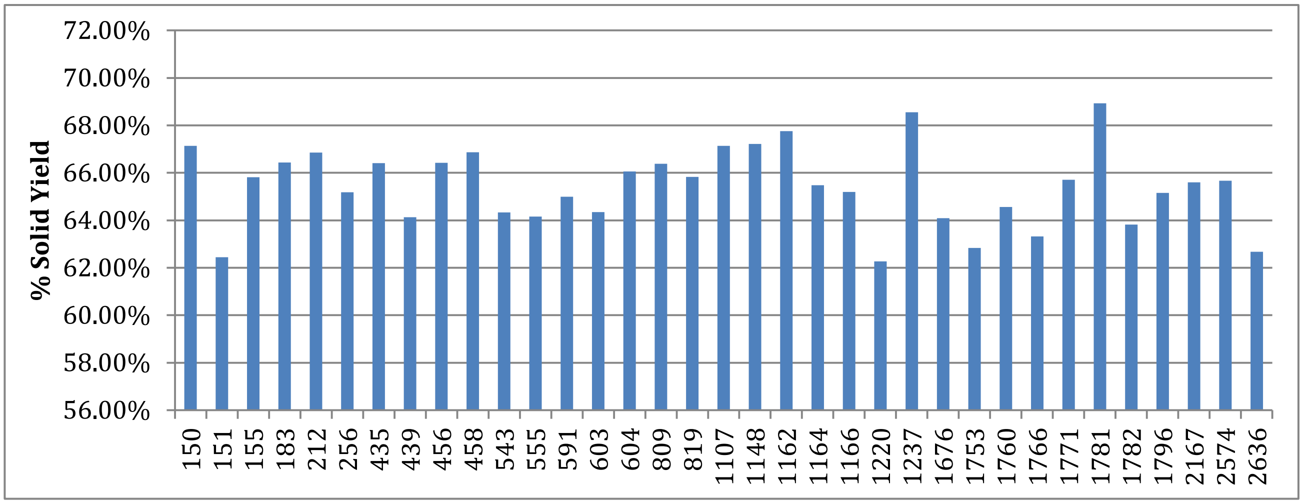


Figure 2: The chemical compositional of different Douglas-fir samples



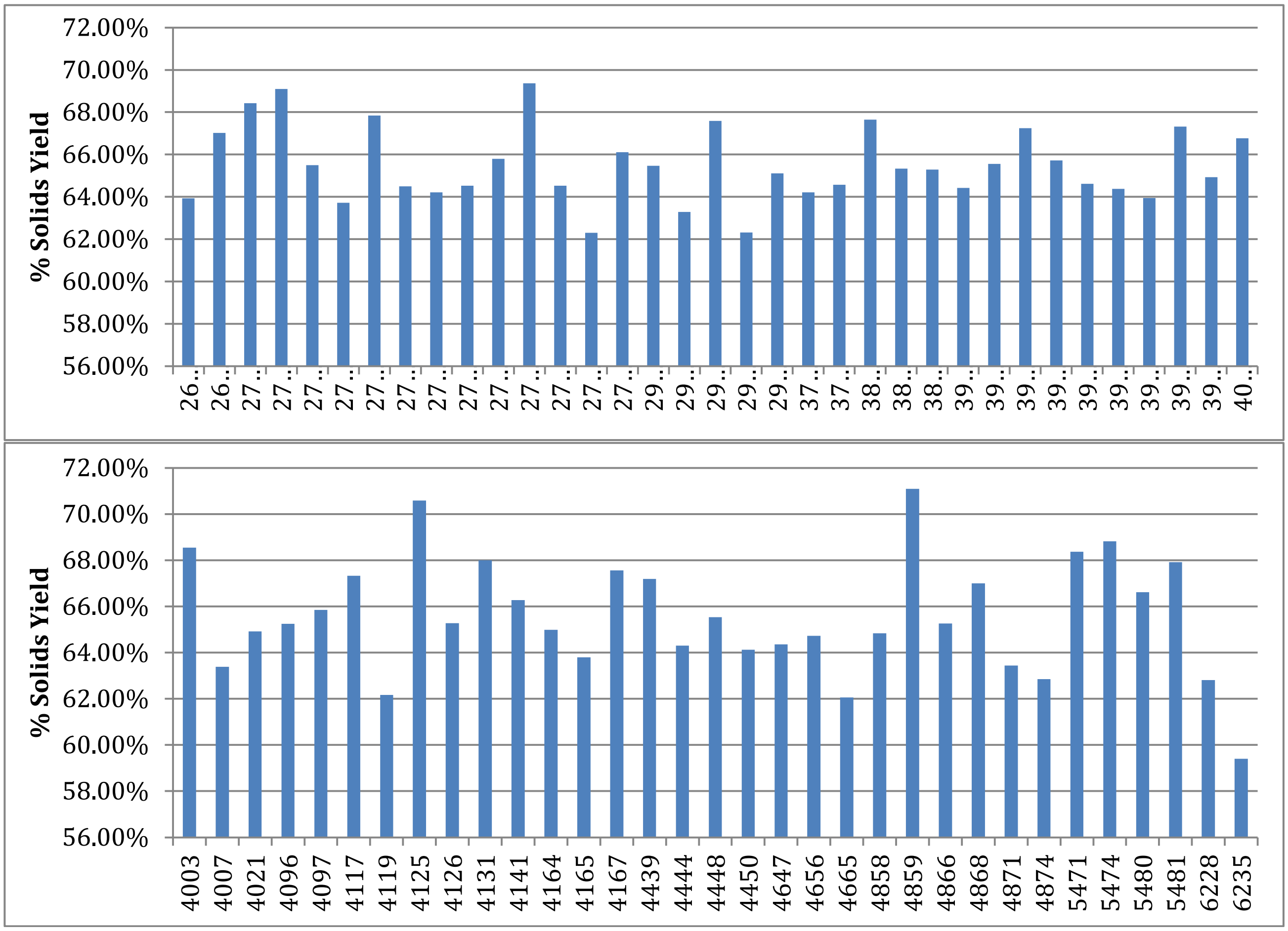
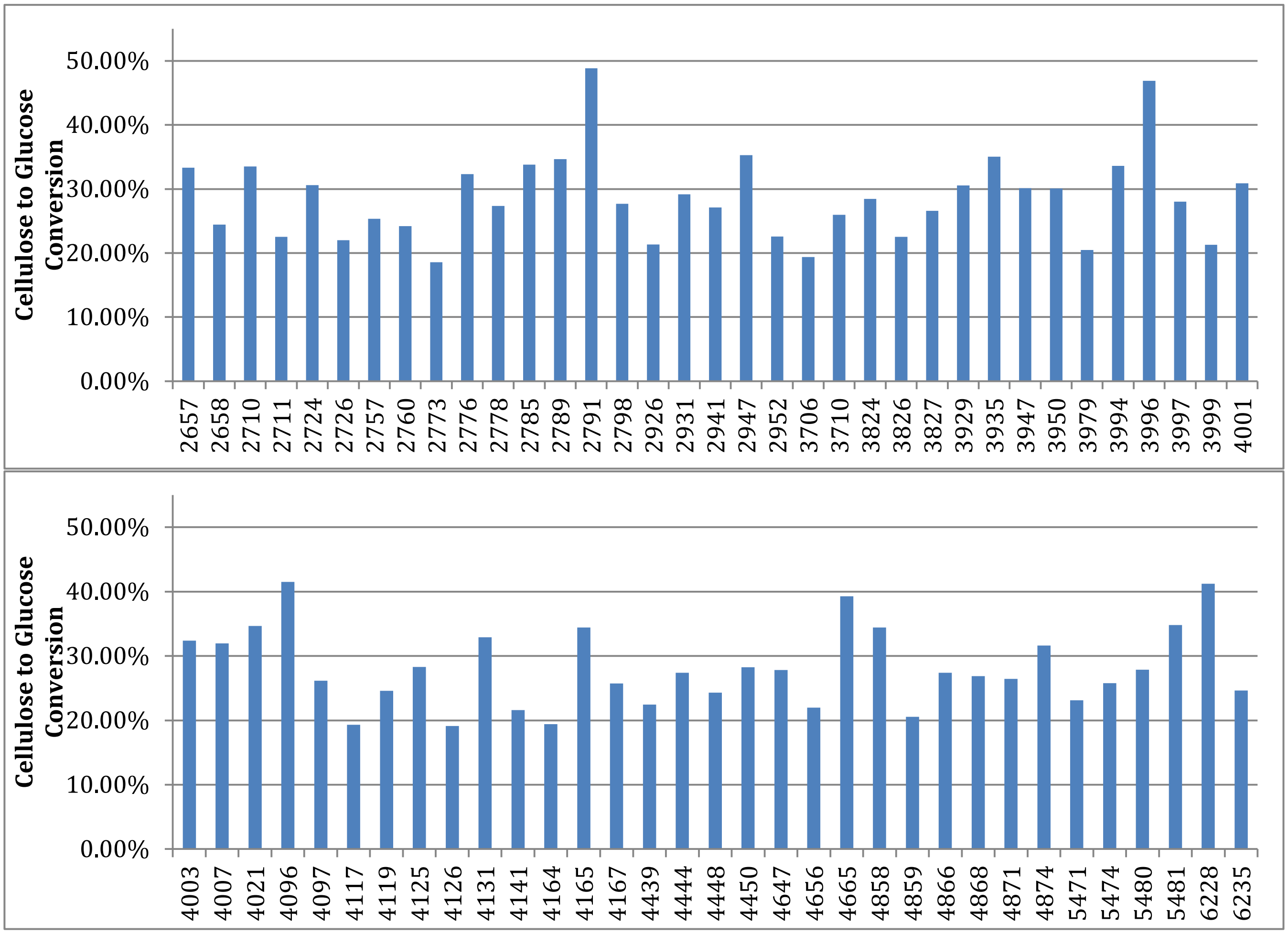


Figure 3: the pretreatment yield of D. fir line after diluted acid pretreatment. (Average: 65.50% Standard Deviation: 1.99%, sample ID is shown on X –axis)

A: Hydrolysis Yield – Dilute Acid (Average: 28.27%, Standard Deviation: 5.96%)



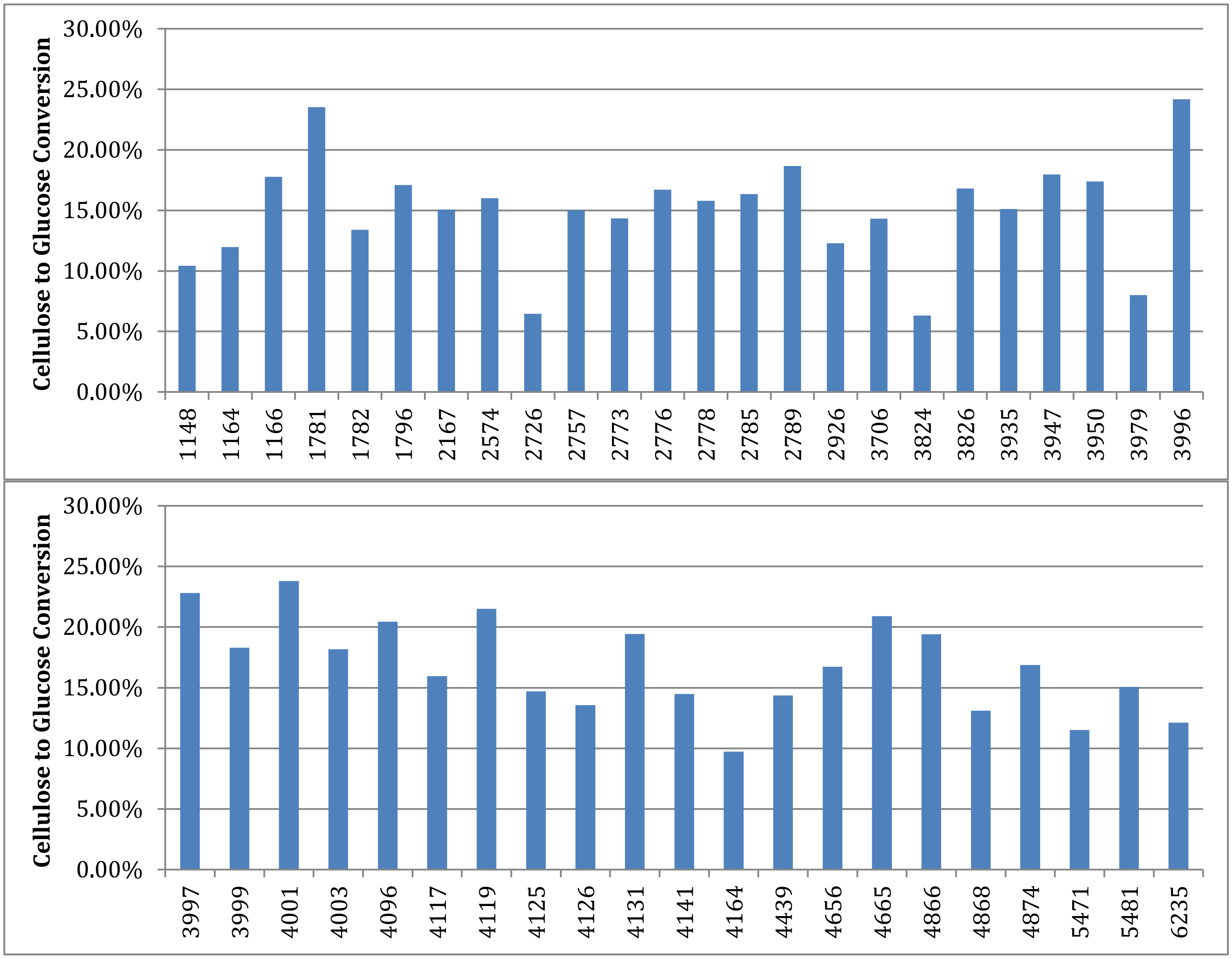

B: Hydrolysis Yield – Alkaline Peroxide (Average: 15.87%, Standard Deviation: 4.21%

Figure 4: Cellulose to glucose conversion yield obtained from cellulase hydrolysis of diluted acid (A) and alkaline peroxide (B) pretreated D. fir family lines (Average: 28.27%, Standard Deviation: 5.96%, sample ID is shown on X –axis)

Recommendations/Conclusions

A systematic research approach is taken to gain a comprehensive understanding of biomass recalcitrance variation of Douglas-fir from several aspects, including chemical composition, pretreatability and digestibility. A large amount of information has been gathered from this first one-and-half year study. The results have shown that the traits associated with biomass recalcitrance were highly variable among different Douglas-fir families. Initial heritability analysis has suggested that genetic control may have a strong influence on some of the “biomass recalcitrance traits” of Douglas-fir families. Douglas-fir family lines with relatively high or low recalcitrance are identified and the information will be provided for subsequent genetic analysis. This research task can help us gain a comprehensive understanding of the recalcitrance of softwood toward biomass conversion. Continued gathering this information from the other Douglas-fir family lines will likely lead to a breakthrough understanding of the underlining genetic control of biomass recalcitrance and provide insight to feedstock selection and breeding for biofuel production.

Physical and Intellectual Outputs

Conference Proceedings and Abstracts from Professional Meetings

Carlos A. Alvarez-Vasco, Xiao Zhang, Keith J. S. Jayawickrama and Karissa B. Garcia “Phenotypic variations of biomass recalcitrance in Douglas fir families”, 35th Symposium on Biotechnology Application on Fuels and Chemicals, April 2013 Portland

Research Presentations

Alvarez-Vasco, C., K. Garcia, K. Jayawickrama, E. Brown and X. Zhang. 2012. High throughput analysis of biomass recalcitrance of Douglas fir families. Panel presentation at NARA 2012 Annual Meeting, Missoula, MT, Sept 13-14, 2012.

Alvarez-Vasco, C., K. Garcia, K. Jayawickrama, E. Brown and X. Zhang. 2012. Variation in Chemical Composition and Biomass Recalcitrance of D. fir Families. Poster presentation at NARA 2012 Annual Meeting, Missoula, MT, Sept 13-14, 2012.

Trainings, Education and Outreach Materials

Pedro Guajardo was awarded the Auvil Scholarship for his work with NARA.

Feedstock Logistics TEAM

Task FL-1: Feedstock Sourcing

Key Personnel	Affiliation
Gevan Marrs	Weyerhaeuser

Task Description

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

Weyerhaeuser will concentrate on the supply chain from young, intensively managed plantations of Douglas-fir in western Oregon and Washington, primarily pre-commercial thinnings. Many of these stands have good access on favorable terrain, but are not currently being utilized; others are on steep terrain, posing greater utilization challenges. Unlike forest residuals from regeneration activities, residuals from pre-commercial thinnings are not currently at roadside. Because of high handling costs, forest owners currently either thin-to-waste or delay thinning, losing potential forest growth. Cost-efficient stump-to-truck collection and comminution systems for young managed stands could contribute significantly to a bioenergy industry. Weyerhaeuser is in strong position to design and implement studies in young managed stands with their well-distributed ownership of Douglas-fir plantations in Oregon and Washington. We will leverage existing harvesting equipment manufacturers to test and document the application of small-tree supply chains that have been successful elsewhere and test modifications of those techniques for our regional conditions and multi-product markets. Weyerhaeuser will: work with the conversion group to match feedstocks from young plantations with required specifications for conversion; document the costs and productivity of existing small-tree supply chains; test and document improvements in the supply chain; estimate potential regional supplies and costs for use in regional biomass modeling; and coordinate with the sustainable production group on long term site productivity studies.

Activities and Results

Although a preliminary analysis of likely feedstock that balances available quantities of Pacific Northwest softwood against cost and quality indicated that forest harvest residuals (“FHR”) were the most plausible candidate for sourcing the NARA project (Figure 1), to get broadly representative materials for all pretreatment pathways with minimum variability and contaminants, Douglas-fir pulp chips were obtained from a pulp mill chip pile and prepared, characterized and distributed to teams as a starting common reference sample.

Eight candidate FHR samples were obtained from operating harvests in western Washington and Oregon. Each sample of about 500 OD pounds was screened to remove fines (which contain a high level of bark and inorganics contaminants), air-dried, fully characterized, and aliquots of chosen samples delivered to pretreatment teams for conversion tests. Figure 2 shows a comparison of one of the major quality attributes—polysaccharides content. As expected, samples with higher bark content tended to have more fines produced, and thus higher fines screening rejects, which of course drives up feedstock cost to conversion mouth.

Quantification of key quality attributes confirmed that not only was there a high degree of variability between FHR samples, there seemed to be considerable opportunity to influence several key factors (bark content, species, and inorganic contamination) once harvesters were aware of desired quality attributes. Figure 3 shows how one main factor—bark content—is broadly correlated to total polysaccharides content, and thus to conversion process yield where fermentation of monosaccharides is the route to biofuels.

A final (largely unexplored to date) controllable factor is how the feedstock is particulated from forest to conversion mouth. Many early NARA feedstock samples were being ground for power combustion and the resulting fines resulted in far too much material loss for biofuels.

Our latest large-scale (~4 OD tons) of NARA FHR reference sample was collected to reduce bark content and fines generation in the woods. Subsequent mill screening and re-sizing of the oversize particles led to a much improved feedstock quality and cost for our subsequent work. (see FS-10 in Figure 3). Table 1 lists the key quality attributes of all NARA feedstocks to date.

In concert with the techno-economic analysis (TEA) task, it was necessary to set a conversion plant scale, which trades off rising feedstock cost against reduced unit capital costs with increasing facility scale. Preliminary FHR availabilities were obtained by county for all NARA states and contiguous 3-to-5 county blocks chosen in each of western Washingtion and Oregon, as well as one in Montana and Idaho, for which the data showed more than 1million BDT/yr FHR availability. A harvesting and hauling model was constructed leading to construction of sourcing curves for western Washington and Oregon (Figure 4).

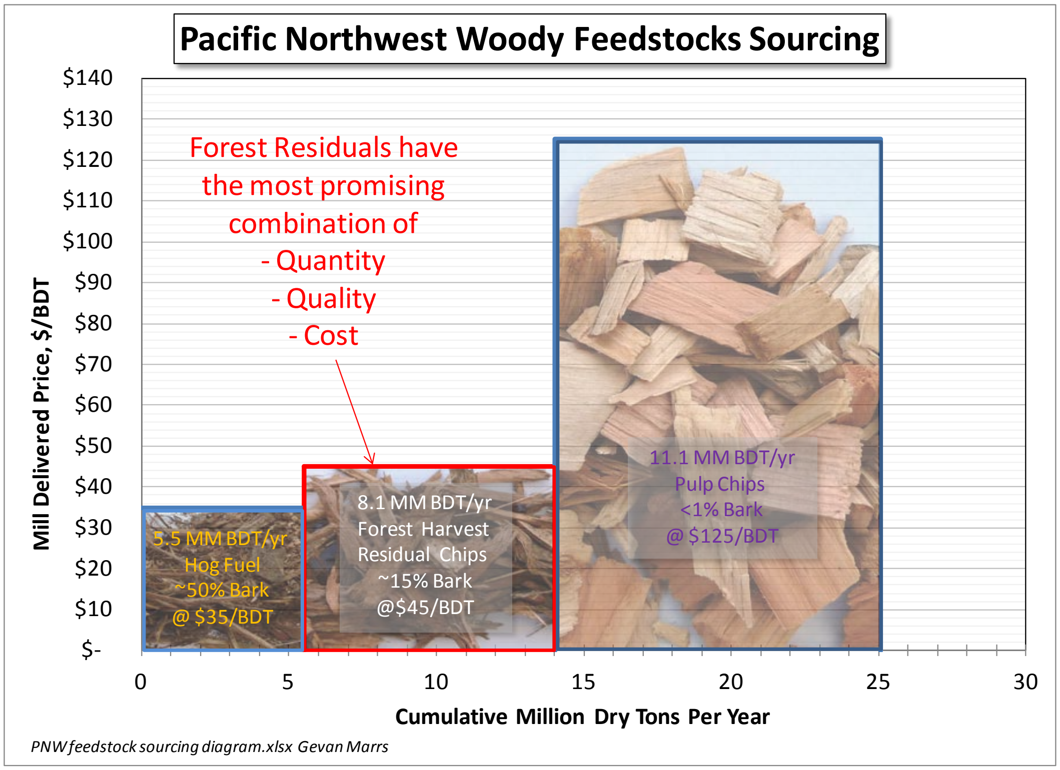
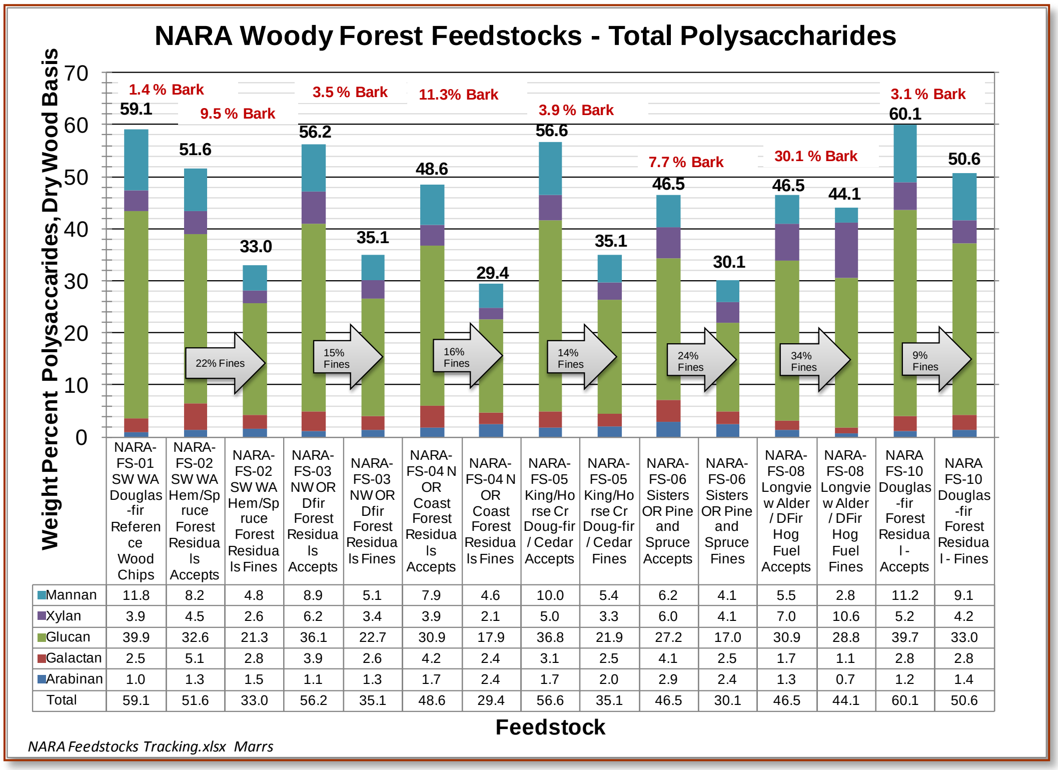


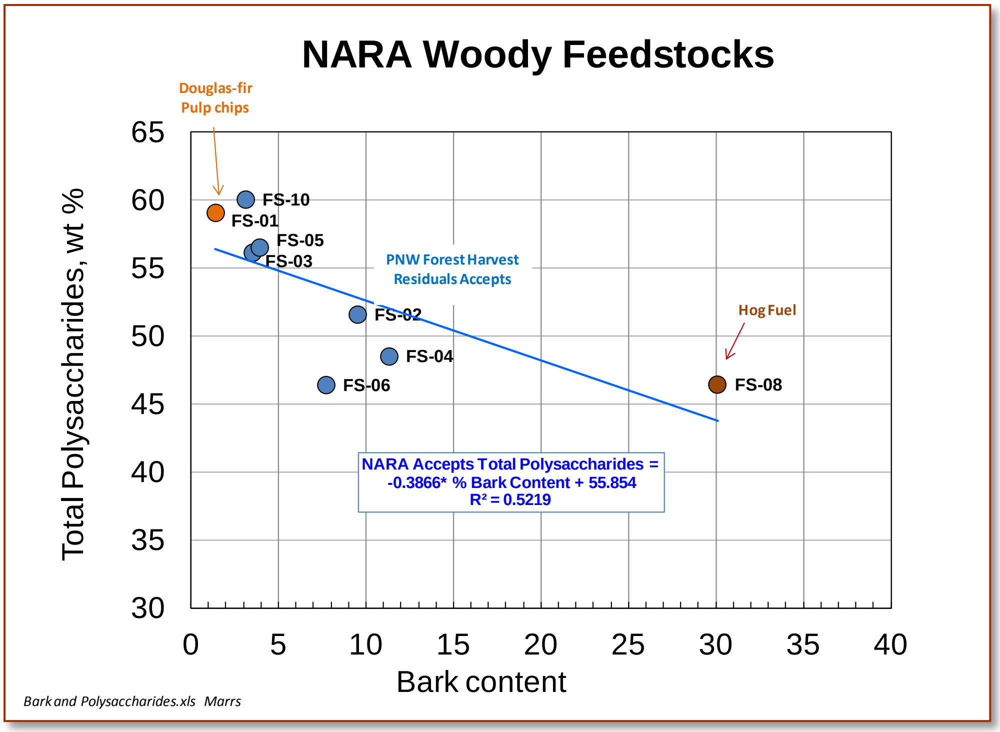
Figure 1. Preliminary estimates of quantities and costs for three basic softwood feedstock categories in the NARA PNW region.



	NARA-FS-01 SW WA Douglas-fir Reference Wood Chips	NARA-FS-02 SW WA Hem/Spruce Forest Residuals Accepts	NARA-FS-02 SW WA Hem/Spruce Forest Residuals Fines	NARA-FS-03 NW OR Dfir Forest Residuals Accepts	NARA-FS-03 NW OR Dfir Forest Residuals Fines	NARA-FS-04 N OR Coast Forest Residuals Accepts	NARA-FS-04 N OR Coast Forest Residuals Fines	NARA-FS-05 King/Horse Cr Doug-fir / Cedar Accepts	NARA-FS-05 King/Horse Cr Doug-fir / Cedar Fines	NARA-FS-06 Sisters OR Pine and Spruce Accepts	NARA-FS-06 Sisters OR Pine and Spruce Fines	NARA-FS-08 Longview Alder / DFir Hog Fuel Accepts	NARA-FS-08 Longview Alder / DFir Hog Fuel Fines	NARA FS-10 Douglas-fir Forest Residual - Accepts	NARA FS-10 Douglas-fir Forest Residual - Fines
Mannan	11.8	8.2	4.8	8.9	5.1	7.9	4.6	10.0	5.4	6.2	4.1	5.5	2.8	11.2	9.1
Xylan	3.9	4.5	2.6	6.2	3.4	3.9	2.1	5.0	3.3	6.0	4.1	7.0	10.6	5.2	4.2
Glucan	39.9	32.6	21.3	36.1	22.7	30.9	17.9	36.8	21.9	27.2	17.0	30.9	28.8	39.7	33.0
Galactan	2.5	5.1	2.8	3.9	2.6	4.2	2.4	3.1	2.5	4.1	2.5	1.7	1.1	2.8	2.8
Arabinan	1.0	1.3	1.5	1.1	1.3	1.7	2.4	1.7	2.0	2.9	2.4	1.3	0.7	1.2	1.4
Total	59.1	51.6	33.0	56.2	35.1	48.6	29.4	56.6	35.1	46.5	30.1	46.5	44.1	60.1	50.6

NARA Feedstocks Tracking.xlsx Marrs

Figure 2. Compilation of one key quality attribute (polysaccharides) for NARA feedstocks sampled to date.



Bark and Polysaccharides.xls Marrs

Figure 3. A key quality parameter—bark content—can vary widely depending upon harvesting and is strongly related to process conversion yield potential.

Table 1. Quality attributes of NARA feedstock samples.

Summary Chemical Analyses NARA Feedstocks										Qualtiy Aspects NAR		
Feedstock	Total Polysacc harides	Hexose Polysacc harides	Pentose Polysacc harides	Lignin, Acid-Insoluble (Klason)	Acid-soluble Lignin	Hot Water Extractives	Ethanol Extractives	Ash	**Total**	Percent Fines Reject	Bark Content	Ash %
NARA-FS-01 SW WA Douglas-fir Reference Wood Chips	59.07	54.2	4.91	26.83	0.28	2.11	1.63	0.20	**90.1**		1.4%	0.20
NARA-FS-02 SW WA Hem/Spruce Forest Residuals Accepts	51.63	45.8	5.82	32.23	0.50	5.22	2.81	1.45	**93.8**		9.5%	1.45
NARA-FS-02 SW WA Hem/Spruce Forest Residuals Fines	32.97	28.9	4.08	44.17	0.87	6.92	4.93	10.48	**100.3**	22.0%	UNK	10.48
NARA-FS-03 NW OR Dfir Forest Residuals Accepts	56.18	48.87	7.31	29.63	0.44	3.71	2.52	1.24	**93.7**		3.5%	1.24
NARA-FS-03 NW OR Dfir Forest Residuals Fines	35.10	30.4	4.73	41.60	0.74	5.55	4.01	15.27	**102.3**	14.8%	UNK	15.27
NARA-FS-04 N OR Coast Forest Residuals Accepts	48.55	43.0	5.59	35.37	0.63	4.91	3.09	2.37	**94.9**		11.3%	2.37
NARA-FS-04 N OR Coast Forest Residuals Fines	29.38	24.9	4.50	44.80	1.10	6.07	6.47	8.47	**96.3**	16.1%	UNK	8.47
NARA-FS-05 King/Horse Cr Doug-fir / Cedar Accepts	56.56	49.8	6.72	29.00	0.43	5.11	2.49	1.65	**95.2**		3.9%	1.65
NARA-FS-05 King/Horse Cr Doug-fir / Cedar Fines	35.10	29.8	5.30	40.30	0.83	7.49	6.16	15.20	**105.1**	13.9%	UNK	15.20
NARA-FS-06 Sisters OR Pine and Spruce Accepts	46.45	37.6	8.89	33.90	0.58	5.43	5.11	2.82	**94.3**		7.7%	2.82
NARA-FS-06 Sisters OR Pine and Spruce Fines	30.12	23.7	6.42	43.47	0.84	7.08	6.29	12.70	**100.5**	24.5%	UNK	12.70
NARA-FS-08 Longview Alder / DFir Hog Fuel Accepts	46.48	38.1	8.37	34.90	0.88	5.52	3.14	5.76	**96.7**		30.1%	5.76
NARA-FS-08 Longview Alder / DFir Hog Fuel Fines	44.10	32.8	11.28	37.73	1.20	5.79	2.48	8.92	**100.2**	33.6%	UNK	8.92
NARA FS-10 Douglas-fir Forest Residual - Accepts	60.09	53.74	6.35	27.53	0.44	3.37	2.14	0.44	**94.0**		3.4%	0.44
NARA FS-10 Douglas-fir Forest Residual - Fines	50.60	44.9	5.66	32.23	0.56	4.34	4.33	1.97	**94.0**	9.0%	UNK	1.97

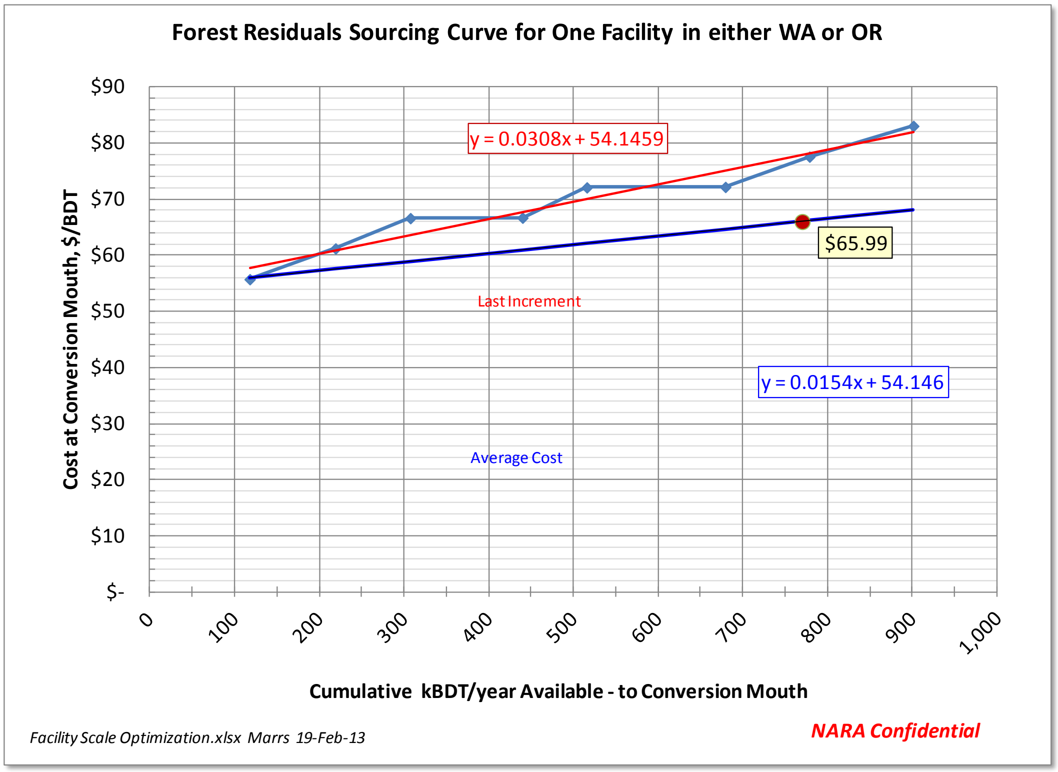


Figure 4. Feedstock sourcing curve for western WA or OR estimates average feedstock cost to a 770 thousand BDT/yr facility at approximately $66/BDT.

Recommendations/Conclusions

Our work to date demonstrates that there is a very high variability in key quality attributes of the most plausible softwood feedstock for a scale biofuels production—forest harvest residuals. Many of this (bark, species, inorganics, particle size) can be impacted by harvesting techniques once the value of doing so is communicated to the harvesting operators. One aspect—optimizing the location, methods, and degree(s) of particle-size reduction and matching to the conversion process sensitivities—is largely unexplored and is not well-defined based upon prior art (like pulp mill procedures, for example). This area has considering financial impact on overall process economics and should be investigate further in coming project years. Additionally, while the initial focus has been on the regions with the highest availability of FHR feedstock at scale, and existing harvesting operations from which to readily obtain samples, work should be directed toward quantifying the opportunity and quality for softwood feedstocks in the Montana corridor.

Physical and Intellectual Outputs

Physical

- Nine different NARA feedstocks were sampled at varying scales (from 500 to 10,000 OD lbs), prepared by size screening, air-dried, fully characterized, and distributed as required for the various pretreatment teams for conversion tests.
- Preliminary feedstock sourcing curves, showing rising cost with conversion facility scale, were prepared and delivered to the TEA group, providing one key manufacturing cost input.

Research Presentations

Marrs, G. 2012. Feedstock sourcing – quality and impacts. Poster presentation at NARA 2012 Annual Meeting, Missoula, MT, Sept 13-14, 2012.

Marrs, G. 2012. Feedstock sourcing – quantity and cost. Poster presentation at NARA 2012 Annual Meeting, Missoula, MT, Sept 13-14, 2012.

Task FL-2: Logistics Decision Support and Improvement

Key Personnel	Affiliation
John Sessions	Oregon State University
Kevin Boston	Oregon State University
Loren Kellogg	Oregon State University
Glen Murphy	Oregon State University

Task Description

We will: 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.

Activities and Results

Task FL-2.1 develops biomass recovery coefficients for Oregon, Washington, Idaho and Montana. A procedure for estimating piled and field distributed biomass has been developed and validated. Packing estimates, the percentage of solid wood in a cubic foot of space, have been collected from 15 sites in western Oregon and Washington. Five additional sites have been identified in eastern Oregon. Sites are being identified in Idaho and Montana in coordination with the University of Montana BBER group. Finally, the prototype of the biomass recovery by species and silvicultural prescription has been developed and prototyped.

Task FL-2.2 develops moisture management strategies and models. We completed the evaluation of six tools (2 acoustic, 2 conductance, and 2 capacitance) for estimating moisture content in three forms of woody biomass (small roundwood logs, chips and hogfuel) in six species (Douglas-fir, ponderosa pine, western hemlock, poplar, garryana oak, and madrone). Criteria for evaluating the tools included accuracy, efficiency and mechanical reliability. All tools required calibration (often species specific). Validation of the three best performing tools in Douglas-fir indicates that the tools are accurate below 35% MC (wet basis). This suggests they could be used for making threshold transportation decisions, i.e., determining when to haul. We have completed a forecasting model for air-drying rates of Douglas-fir in Oregon. Analysis of data from 3000 truckloads of forest harvesting residuals delivered to a cogeneration plant at Eugene, Oregon indicates moisture content varies as a function of season of harvest and season of piling. Preliminary data analysis also suggests that a significant number of truck loads are being delivered underweight. Low bulk density and large trailer access may be limiting transportation efficiency.

Task FL-2.3 refines collection and transport models for regional modeling. A decision support model for determining the optimal grinding/chipping equipment/trucking configuration for comminution has been completed. Field studies needed to develop and validate this model included mobile chipping and stationary grinding at 12 sites in Oregon and Washington. Actual grinding and chipping utilization rates varied widely (20% to 80% utilization) depending upon truck availability and road configurations (Figure 1). We found that each 10% increase in grinder/chipper utilization could reduce costs $0.5-$3.0/dry ton (Figure 2). Upper limits on grinder utilization depended upon the road and landing configuration. Simulations using this decision support model will be used to inform the Timber Supply Model.

Task FL-2.4 evaluates chipping and grinding production to meet alternative feedstock specifications. Grindings from eight geographic areas in western Washington, western Oregon and eastern Oregon were collected and transported to the Weyerhaeuser Technical Center for characterization by the Feedstock Sourcing team. One additional example of chips from a mobile chipper operating in Douglas-fir forest harvest residuals was sent to the Forest Products Laboratory. In addition, forest residuals from 55 sites were characterized under a companion study at Oregon State University (Smith, Sessions, et al. Forest Products J, manuscript accepted). Study of residue characteristics from the 55 samples (Figure 3) is being used to design structured tests for Summer, 2013 to increase uniformity, reduce moisture content, reduce ash content, and increase dry bulk density. Bark and ash are concentrated in the Fines Fraction.

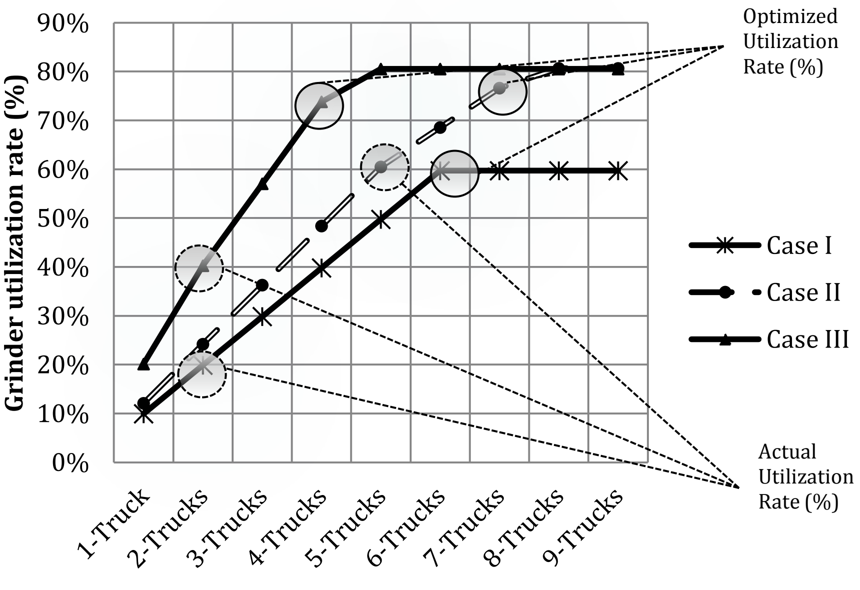


Figure 1. Grinder utilization rate as a function of truck availability showing actual utilization and utilization under the combination of trucks that provided the lowest comminution plus transportation costs (Zamora et al., manuscript in review)

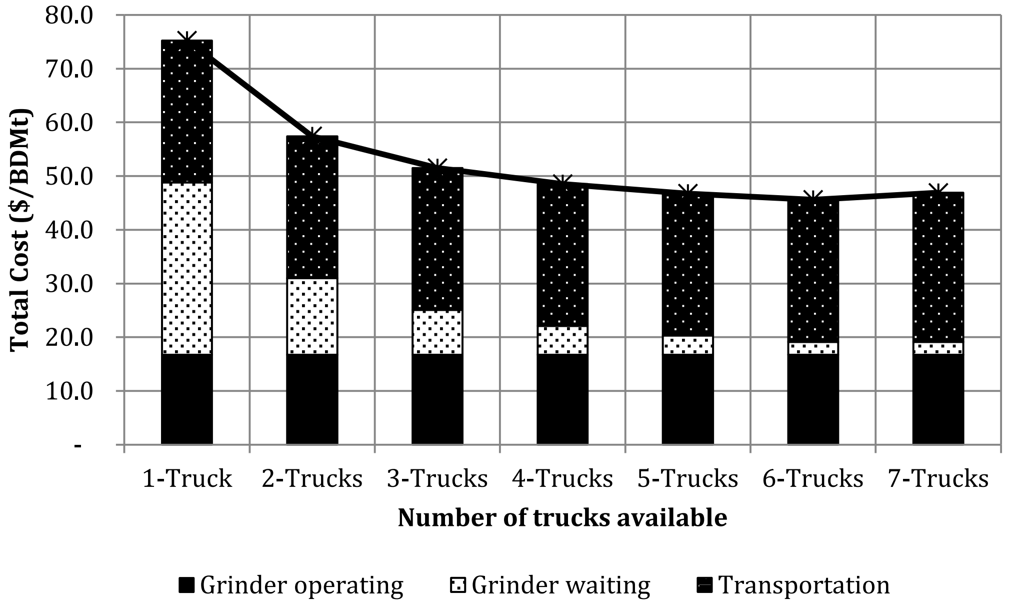


Figure 2. Delivered wood cost ($/dry metric tonne) for Case II as a function of truck availability (Zamora et al., manuscript in review)

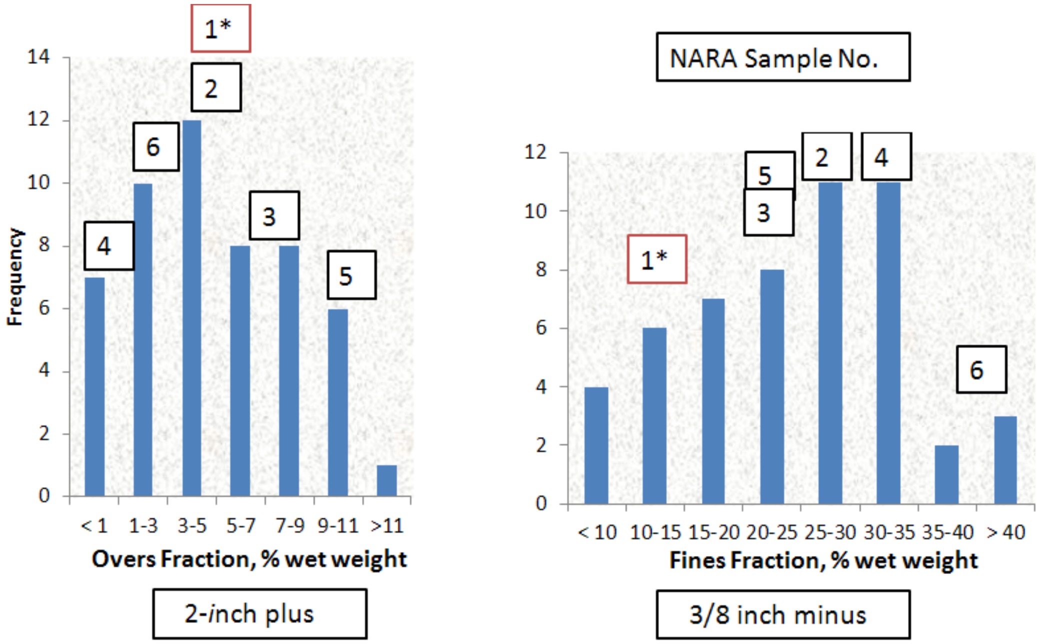


Figure 3. Example of wide variation in ground and chipped materials from forest harvest residue operations in Washington and Oregon (Smith, Sessions, et al, manuscript in review). Data points from six of the NARA sites illustrate the similar variation in materials within the NARA samples and the larger data set.

Recommendations/Conclusions

Currently, we are approximately 40% complete with our survey of volume and locations of piles. In the next quarter, we will focus our efforts in western Washington and east of the Cascades. We are seeing a significant variability by logging system with shovel logging producing more, smaller piles scattered throughout the unit. As expected, cable logging produces fewer piles near the landings.

Managing moisture is one of the keys to improving the economic feasibility of woody biomass use. Transportation costs and market values of woody biomass are strongly linked to the amount of moisture in the woody biomass. We have developed drying rate models for small logs for two species that have been stored in-forest. We have assembled data that should allow prediction of drying rates for biomass residue piles in western Oregon. We have identified tools that could be used in-forest for determining when moisture contents have dropped below 35% (wet basis), a potential transportation decision threshold. Our emphasis next year will be on determining the effect of harvesting season on initial residue moisture contents, the effect of residue pile design on drying rates, and building an economic model that can tell us the optimal storage time balancing cost savings against capital, interest and risk costs.

Our next steps are to complete the biomass recovery coefficients and collection and transportation cost models for the feedstock supply model. By August we will be able to provide recovery coefficients, comminution and transportation costs to the Feedstock Supply modeling group that will be used to develop supply curves for forest residuals to alternative locations in the NARA region.

The decision support models show the economics of comminution and transport rely heavily on operational planning, moisture management and access for large trailers. Next year we will complete the moisture management models, implement structured tests to determine the effects of grinder grate (screen size), moisture content, chipper bit configuration, and piece size on feedstock characteristics, and investigate alternatives for increasing dry bulk density.

Physical and Intellectual Outputs

Physical

- Fifteen sites were sampled to inventory the volume and location of piles
- 156 logs from 6 species were collected and their moisture content repeatedly measured over a five month period using 6 tools to identify suitable tools for in-forest moisture management
- Approximately 50 tons of Douglas-fir and 35 tons of hybrid poplar were bundled and air-drying rates determined as a function of drying location within Oregon, length of drying time, and season when drying began.
- A database of 3000 trailer loads of forest residuals were analyzed for moisture content as a function of harvest and piling variables.
- Eight 500 pound forest residuals from different geographic zones in Oregon and Washington were collected and sent to the Weyerhaeuser Technology Center (WTC) for characterization
- One 14,000 pound forest harvest residue of Douglas-fir was sent to WTC in January, 2013 to be used as the reference Douglas-fir sample for laboratory testing
- A Decision Support System was developed that forest planners can use for optimizing comminution and transport decisions.
- 11 manuscripts were developed by project personnel to reach professional audiences.

Refereed Publications (accepted or completed)

Smith, D., J. Sessions, K. Tuers, D. Way and J. Traver. 2013. Characteristics of forest derived woody biomass collected and processed in Oregon. Forest Products J. Manuscript accepted.

Kim, D-W. and G. Murphy. 2012. Forecasting air drying rates of Douglas-fir and hybrid poplar biomass in Oregon, USA. International Journal of Forest Engineering. Manuscript accepted.

Sessions, J., K. Tuers, K. Boston and R. Zamora. 2013. Pricing Forest Biomass for Power Generation. West J. of Applied Forestry. 28(2):51-56.

Zhang, C., J.Y. Zhu, R. Gleisner, and J. Sessions. 2012. Fractionation of Forest Residues of Douglas-fir for Fermentable Sugar Production by SPORL Pretreatment. BioEnergy Research. 5(4):978-988. DOI: 10.1007/s12155-012-9213-3.

Long, J. and K. Boston. An evaluation of measurement techniques for estimating the volume of piled logging residue. Western Journal of Applied Forestry. Manuscript in review.

Beck, S. and J. Sessions. Ant Colony Optimization for Forest Road Access Decisions for non-conventional products. Croation J. of Forest Engineering. Manuscript in review.

Clark, J., Sessions, J. and R. Zamora. Optimizing Knife Change Times for Forest Biomass Chipping Operations. Biomass and Bioenergy. Manuscript in review, Zamora, R., P. Adams, and J. Sessions. Ground-based thinning on steep slopes in Western Oregon: Soil compaction and disturbance effects. Western J. of Applied Forestry. Manuscript in review.

Zamora, R., K. Boston, and J. Sessions. Activity-Based Stochastic Simulation of Mobile Chipper Productivity.European J. of Operational Research. Manuscript in review.

Zamoa, R., J. Sessions, and G. Murphy. Economic impact of truck-machine interference in forest biomass recovery operations on steep terrain. Forest Products J. Manuscript in review.

Leu, Shao-Yuan, R. Gleisner, R., J.Y. Zhu, J. Sessions, and G. Marrs. Robust Enzymatic Saccharification of a Douglas-fir forest harvest residue by SPORL. Submitted to Biomass and Bioenergy.

Research Presentations

Posters:

Zamora, R., K. Boston, and J. Sessions. 2012. Activity-Based Stochastic Simulation of Mobile Chipper Productivity. Prepared for 2012 Annual Meeting for the Northwest Advanced Renewables Alliance (NARA), Sept. 13-14, Missoula, MT. Also presented November 13, 2012 at Northwest Bioenergy Research Symposium, Seattle, WA.

Zamora, R. and J. Sessions. 2012. Economic Optimization of Forest Biomass Processing and Transport: A Decision Support System. Prepared for 2012 Annual Meeting for the Northwest Advanced Renewables Alliance (NARA), Sept. 13-14, Missoula, MT. Also presented November 13, 2012 at Northwest Bioenergy Research Symposium, Seattle, WA.

Zamora, R., B. Flint, J. Sessions, L. Kellogg, and P. Adams. 2012. Ground-Based Thinning on Steep Slopes in Oregon: Productivity, Economics and Soil Compaction Effects. Prepared for 2012 Annual Meeting for the Northwest Advanced Renewables Alliance (NARA), Sept. 13-14, Missoula, MT. Also presented November 13, 2012 at Northwest Bioenergy Research Symposium, Seattle, WA.

Zamora, R. and J. Sessions. 2012. Characteristics of NARA Forest Residue Sites. Prepared for 2012 Annual Meeting for the Northwest Advanced Renewables Alliance (NARA), Sept. 13-14, Missoula, MT. Also presented November 13, 2012 at Northwest Bioenergy Research Symposium, Seattle, WA.

Zhu, J, Y., C. Zhang, J. Sessions, and R. Gleisner. 2012. Fractionation of Douglas-fir Forest Residues for Efficient Sugar Production. Prepared for 2012 Annual Meeting for the Northwest Advanced Renewables Alliance (NARA), Sept. 13-14, Missoula, MT.

Buffum, M., G. Murphy, F. Belart, F. Becerra and B. Do. 2012. Assessing moisture content in biomass piles. NARA Summer Undergraduate Research Experience. Washington State University, Pullman WA. August 2.

Do, B., G. Murphy, F. Belart, F. Becerra and M. Buffum. 2012. Assessing risks of arson in biomass piles. NARA Summer Undergraduate Research Experience. Washington State University, Pullman WA. August 2.

Presentations:

Sessions, J. 2012. Perspectives and Priorities for Bioenergy Research at Oregon State University. Presentation at Northwest Bioenergy Research Symposium, Seattle, WA. November 14. Invited Opening Plenary Presentation.

Beck, S. and J. Sessions. 2012. Ant Colony Optimization for Road Maintenance Decisions. Presentation at Council on Forest Engineering. New Bern, North Carolina. September 10-11.

Belart, Francisca and G. Murphy. 2012. Forecasting and monitoring moisture of woody biomass in Ireland and Oregon. Presentation at Council on Forest Engineering. New Bern, North Carolina. September 10-11.

Murphy, Glen. 2012. Forecasting and monitoring moisture of woody biomass in Ireland and Oregon. Precision Forestry Symposium, Mount Gambier, Australia, 28th March 2012.

Sessions, John. 2012. Project overview to Dan Biggs, Tillamook County Economic Development Council and Assistant OSU Provost, March 22.

Sessions, John. 2011. Invited overview of the NARA project to a group of forest industry leaders and the Presidents of Oregon State University and University of Oregon in Corvallis on September 29, 2011.

Sessions, John. 2011. Invited overview of the NARA project to Oregon delegation staffers at a meeting at the College of Forestry on December 5, 2011.

Other Publications

Zamora, R. 2011. Production and time study of the BRUKS mobile chipper in southwest Oregon. Report prepared and submitted to industrial cooperator.

Thesis and Dissertations

Becerra, Fernando. 2012. Evaluation of Six Tools for Estimating Woody Biomass Moisture Content. MS thesis. Completed December 13, 2012. Glen Murphy, Advisor.

Kim, Dong-Wook. 2012. Modeling air-drying of Douglas-fir and hybrid poplar biomass in Oregon. MS thesis. Completed June 6, 2012. Glen Murphy, Advisor

CONVERSION

Pretreatment Team

Task C-P-1: Pretreatment to Overcome Recalcitrance of Lignocellulose

Key Personnel	Affiliation
Junyong (J.Y.) Zhu	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., Applied Microbiology and Biotechnology, 86:1355-1365, 2010; Tian et al. Bioresource Technology 101:8678-8685; Lan et al., Bioresource Technology, 127:291-297, 2013). The major work for the proposed study is to demonstrate the performance of the SPORL using Douglas-fir as well as Douglas-fir forest residues with relatively high lignin contents, and its scalability at two pilot plant facilities for 1000 gallon biojet fuel production. The focus of the study is on low cost and low value Douglas-fir forest residues to improve economics and sustainability. The *specific objectives* are: (1) to optimize SPORL pretreatment conditions for Douglas-fir 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 ten tons 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 biojet fuel production and lignin co-product development form SPORL hydrolysates and lignin fractions.

Activities and Results

We have completed SPORL pretreatment optimization using clean Douglas-fir wood chips (FS-01) at a laboratory bench scale of 150 g. We conducted pretreatment optimization at two temperatures of 180ºC and 165ºC. The main findings were pretreatment at low temperature of 165ºC with longer time of 75 min significantly reduced inhibitor formation under equivalent cellulose saccharification efficiency and sugar yield. We have examined the experimental data using a kinetic model we developed previously (Zhu et al., *Process Biochemisty*, **47**:785, 2012) and identify the difference in reaction kinetics between hemicellulose hydrolysis and sugar degradation. At the optimal condition (165ºC for 75 min, acid and sodium bisulfite charges on wood of 2.2 and 10wt%, respectively, with liquid to wood ratio of 3:1) identified based on total sugar yield as well as inhibitor formation. We achieved total monomeric sugar yield of 505.2 g/kg wood with total furan concentration in the hemicellulosic sugar stream of approximately 2 g/L. Enzymatic hydrolysis glucose yield is 86% theoretical at a cellulase loading of 15 FPU/g glucan or 0.058 mL/g solid substrate of CTec2.

Several pretreatment runs at 2 kg scale were produced at the identified optimal condition. The resultant solid substrate and the pretreatment hydrolysate that contains the hemicellulosic sugars and lignosulfonate were sent to Gevo for fermentation and to Weyerhaeuser for lignin co-product development.

We also studied the effect of bark on SPORL pretreatment. We investigated the potential to fractionate bark and ash by using physical fractionation based on size. We found that physical fractionation is very effective with excellent selectivity to reduce bark and ash content in the forest residues (Figure 1; Zhang et al., BioEnergy Research 5(4):978). For the sample studied, a 2 mesh screen can retain 60% of the total mass but only contain approximately 30-35% of the bark and ash. When comparing chipping and ground in harvesting forest residues, it appears that physical fractionation is more effective for grounding obtained forest residue to reduce bark and ash content. This is critical to reduce dead load shipping and processing and upgrading feedstock to improve economics.

We also completed SPORL pretreatment optimization using a Douglas-fir forest residue (FS-03) at laboratory bench scale of 150 g. This residue has very high lignin content of 32.3% with glucan content of only 37.7% based on our laboratory analysis. The optimal condition based on total sugar yield as well as inhibitor formation were same as using clean Douglas-fir wood chips, i.e. 165ºC for 75 min, acid charges on wood of 2.2, but sodium bisulfite charge increased to 12%. We achieved enzymatic hydrolysis glucose yield of 345 g/kg or equivalent to 82.3% theoretical at CTec 2 dosage of 15 FPU/g glucan. Total furan formation was 4.5 g/L. This level of furan concentration is still manageable for fermentation. A mass balance analysis is shown in Figure 2.

Again, several pretreatment runs at 2 kg scale were produced at the identified optimal condition. The resultant solid substrate and the pretreatment hydrolysate that contains the hemicellulosic sugars and lignosulfonate were sent to Gevo for fermentation.

We also made a significant finding: using an elevated pH 5.5 or higher can significantly enhance enzymatic hydrolysis of lignocelluloses, especially substrates produced form SPORL pretreatment (Figure 3; Lou et al., ChemSusChem, DOI: 10.1002/cssc.201200859). This contradicts a well-established and widely accepted concept of “optimal pH of 4.8 – 5.0” that has been exclusively practiced in numerous laboratories throughout the world. By using pH 5.5, we can reduce cellulase loading by approximately 50%.

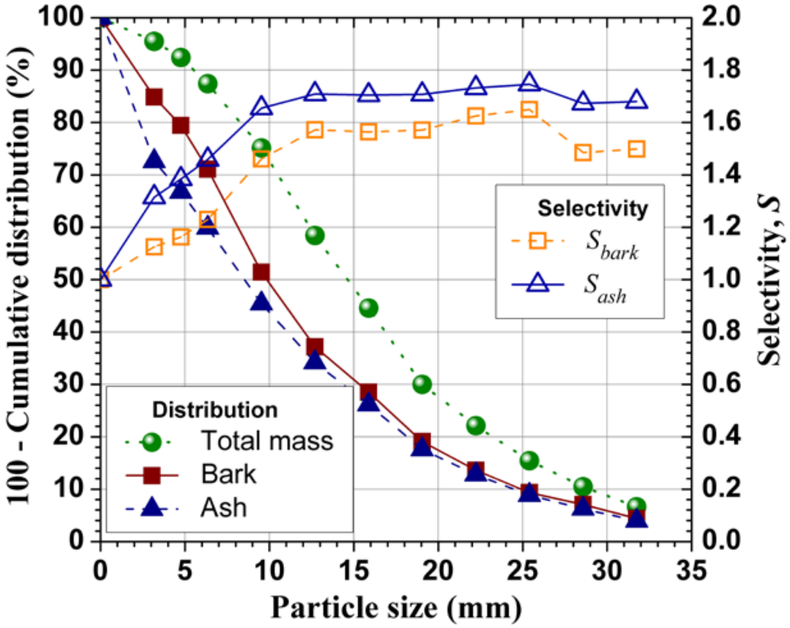


Figure 1. Accumulative (from the largest fraction) distributions of oven dry total mass, bark, and ash and sieving selectivities over bark and ash by particle/chip size of a chipped Douglas-fir forest residues.

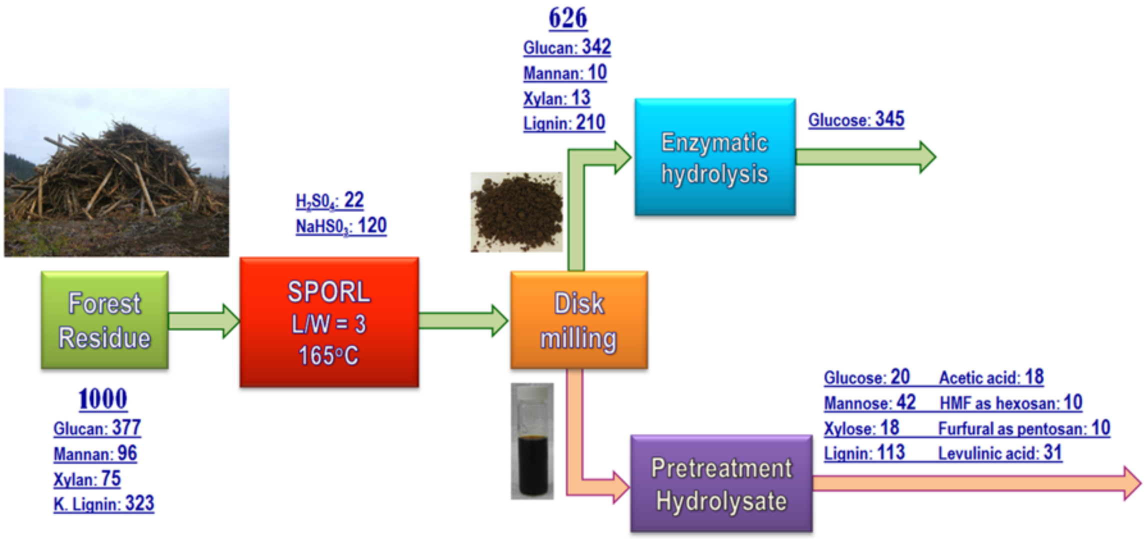


Figure 2 Overall mass balance of the optimal run at T = 165ºC, t = 75 minutes, B = 12 %, and A = 2.2 % with liquid to solid ratio of 3:1. All numbers are expressed in grams.

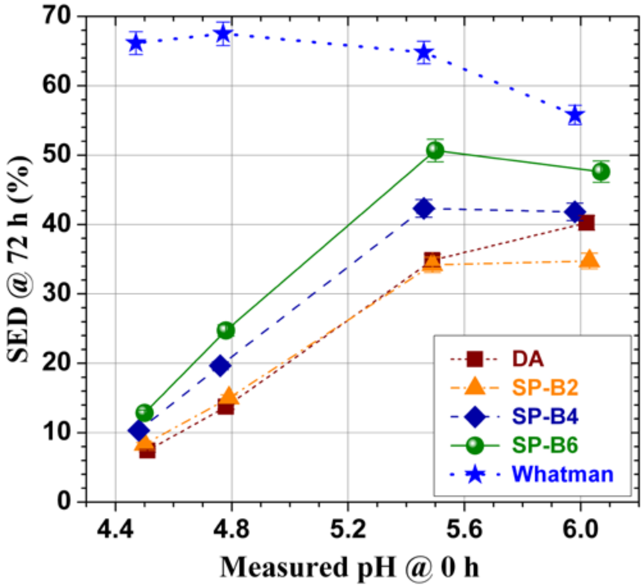


Fig. 3 Effects of pH on enzymatic saccharification of four lignocelluloses and Whatman paper. SP stands for SPORL and DA stands for dilute acid pretreatment

Recommendations/Conclusions

Based on the results obtained so far that include sugar yield, enzymatic saccharification efficiency, cellulase loading, inhibitor formation, and fermentation data from Gevo at the relatively low pretreatment temperature of 165ºC, we are very confident that SPORL pretreatment is very effective to deal with Douglas-fir forest residue, a very recalcitrant feedstock, but is one of the only two most viable and lost cost feedstock based on a recent National Council Report (*http://www.nap.edu/catalog.php?record_id=13105*). In our initial proposal, Douglas-fir was selected as the main feedstock for the project. With the compelling data obtained, we are shifting our focus to forest residues to make this project much closer to a realistic forest biorefinery.

In the next step, we will work on the following issues:

(1) We will scale up studies to a 50 kg scale at the Forest Products Laboratory. This is critical to achieving the project goal of 1000 gallons of biojet fuel production
(2) We will further improve pretreatment to reduce inhibitor formation and improve hemicellulosic sugar yield. We have several novel approaches to address this issue.
(3) We will coordinate and work with our partners to move the project forward on several fronts: feedstock upgrade with Weyerhaeuser and Oregon State University; fermentation with Gevo; lignin co-product development with Weyerhaeuser and Washington State University, etc.
(4) We will look into several opportunities for a 10 ton pretreatment facility and site.

Physical and Intellectual Outputs

Physical

- 6 kg pretreated FS-01 and FS-03 solid samples were sent to GEVO
- 5 L hemicellulosic sugar streams from FS-01 and FS-03 were sent to GEVO
- 400 g lignosulfonate were sent to Weyerhaeuser
- 400 g hydrolysis lignin residue were sent to Weyerhaeuser
- 20 g hydrolysis lignin residue were sent to Washington State University
- 50 kg scale demonstration site was established

Refereed Publications (accepted or completed)

Zhang, C., C.J. Houtman, J.Y. Zhu, 2013. Maximize Enzymatic Saccharification and Minimize Sugar Degradation in SPORL Pretreatment of Douglas-fir at a Low Temperature: A Kinetic Approach. *Bioresource Technology* (in Preparation).

Leu, S.-Y., Gleisner, R., J.Y. Zhu**,** Sessions, J., Marrs, G., 2013. Robust Enzymatic Saccharification of a Douglas-fir Forest Harvest Residue by SPORL, *Biomass and Bioenergy* (submitted)

Lou, H., J.Y. Zhu, T.Q. Lan, H. Lai, and X. Qiu., 2013. pH-Induced Lignin Surface Modification to Reduce Nonspecific Cellulase Binding and Enhance Enzymatic Saccharification of Lignocelluloses. *ChemSusChem*, DOI: 10.1002/cssc.201200859

Leu, S-Y. and J.Y. Zhu. 2013. Substrate-Related Factors Affecting Enzymatic Saccharification of Lignocelluloses: Our Recent Understanding. *BioEnergy Research*. DOI: 10.1007/s12155-012-9276-1

Zhang, C., J.Y. Zhu, R. Gleisner, and J. Sessions. 2012. Fractionation of Forest Residues of Douglas-fir for Fermentable Sugar Production by SPORL Pretreatment. *BioEnergy Research*. 5(4):978-988. DOI: 10.1007/s12155-012-9213-3

Conference Proceedings and Abstracts from Professional Meetings

Zhang, C., Mann, D., Zhu, J.Y., Sessions, J., Marrs, G. 2013. Upgrading Forest Residues of Douglas-fir through Physical Fractionation for Fermentable Sugar Production. Abstract of the 35th Symposium on Biotechnology for Fuels and Chemicals, Portland, OR, April 29 – May 2.

Leu, S.-Y., Zhang, C., Zhu, J.Y., 2013. Sugar production from forest residue of Douglas-fir using SPORL pretreatment: in comparison with bark-free wood chips. Abstract of the 35th Symposium on Biotechnology for Fuels and Chemicals, Portland, OR, April 29 – May 2.

Lou, H., J.Y. Zhu, T.Q. Lan, H. Lai, and X. Qiu., 2013. pH-Induced Lignin Surface Modification to Reduce Nonspecific Cellulase Binding and Enhance Enzymatic Saccharification of Lignocelluloses. Abstract of the 35th Symposium on Biotechnology for Fuels and Chemicals, Portland, OR, April 29 – May 2.

Research Presentations

Zhang, C., Mann, D., Zhu, J.Y., Sessions, J., Marrs, G. 2013. Upgrading Forest Residues of Douglas-fir through Physical Fractionation for Fermentable Sugar Production. Oral presentation at 35th Symposium on Biotechnology for Fuels and Chemicals, Portland, OR, April 29 – May 2.

Lou, H., J.Y. Zhu, T.Q. Lan, H. Lai, and X. Qiu., 2013. pH-Induced Lignin Surface Modification to Reduce Nonspecific Cellulase Binding and Enhance Enzymatic Saccharification of Lignocelluloses. Poster presentation at 35th Symposium on Biotechnology for Fuels and Chemicals, Portland, OR, April 29 – May 2.

Leu, S.-Y., Zhang, C., Zhu, J.Y., 2013. Sugar production from forest residue of Douglas-fir using SPORL pretreatment: in comparison with bark-free wood chips. Poster presentation at the 35th Symposium on Biotechnology for Fuels and Chemicals, Portland, OR, April 29 – May 2.

Zhu, J.Y., 2012. Biofuel Production from Woody Biomass: Net Energy Production Evaluations. Plenary session of 3rd International Energy Congress, 2012 AIChE Annual Meeting, Pittsburgh, PA, October 28-Nov. 2.

Zhang, C., Zhu, J.Y., Sessions, J., 2012. Fractionation of Forest Residues of Douglas-fir for Fermentable Sugar Production by SPORL Pretreatment, presented at the 2012 AIChE Annual Meeting, Pittsburgh, PA, October 28-Nov. 2

Zhu, J.Y., 2012. What we learned from (sulfite) pulping – sulfite pretreatment (SPORL) process", Canadian National Science and Engineering Research Council (NSERC) Bioconversion Network Pretreatment Workshop, Invited Plenary Presentation, University of British Columbia, Vancouver, BC, Canada, June 4-6

Zhu, J.Y. 2012. On sulfite pretreatment to overcome recalcitrance of lignocelluloses (SPORL) for robust bioconversion of woody biomass. Presented at the 243rd ACS National Spring Meeting, San Diego, CA, March 25-29, 2012.

Zhu, J.Y., 2012. Fundamentals and practices for efficient production of biofuel from lignocelluloses. Presented at the 243rd ACS National Spring Meeting, San Diego, CA, March 25-29, 2012.

Zhu, J.Y., C. Zhang, S-Y. Leu and R. Gleisner. 2012. Sugar production from Douglas-fir by SPORL pretreatment. Poster presentation at NARA 2012 Annual Meeting, Missoula, MT, Sept 13-14, 2012.

Task C-P-2: Dilute Acid Pretreatment of Softwood and Lignin Products Development

Key Personnel	Affiliation
Xiao Zhang	Washington State University

Task Description

Task C-P-2.1 is to assist in optimizing large scale pretreatment and lignin product development. In our earlier work, we demonstrated a novel lignin conversion pathway to produce high value phenolic compounds and carboxylic acids. A PCT patent is currently in application “METHODS TO CONVERT LIGNIN TO PHENOLIC AND CARBOXYLATE COMPOUNDS” US-842432-01-US-PCT and has been licensed to intellectual ventures. As shown in Figure 1, lignin can be oxidized by organic peroxyacids or hydrogen peroxide in the presence of certain catalysts to a mixture of low molecular weight phenolic compounds and dicarboxylic acids under mild conditions (atmospheric pressure and reaction temperature up to 90°C). This oxidative lignin degradation method can convert lignin to a number of valued added products and precursors for adhesive, animal feed, polymer and hydrocarbon fuel applications.

Task C-P-2.2 is to study diluted acid pretreatment of Douglas-fir wood and forest residues. This is a new task started in August 2012. There are three components to this task:

1. We will optimize diluted acid pretreatment for carbohydrate and lignin recovery.
The reference Douglas-fir wood chips and residues will be pretreated by diluted acid at a series of conditions (temperature 180—230°C, acid concentration 1-4% and time 30 -90 minutes, different wood to liquid ratios, etc.). The mass balance before and after pretreatment will be determined. The hydrolysability of pretreated substrates will be investigated by using Novozymes Cellic® CTec-II enzyme preparation. The optimized pretreatment conditions will be determined based on both mass recovery yield and substrate hydrolysability.

2. We will prepare pretreated substrate and hydrolysate for Gevo fermentation testing.
Once the optimized pretreatment conditions are identified, we will prepare pretreated substrates and hydrolysates from the four softwood samples for Gevo to test fermentation for butanol production. We will assist Gevo to determine the potential inhibitory compounds present in diluted acid pretreated substrate/hydrolysate and optimize the fermentation parameters.

3. We will prepare and separate diluted acid lignin for co-products development.
Separate 300 grams of diluted acid lignin will be prepared from pretreated substrate and provided to the co-products teams to test potential applications. We will work with the co-products team on lignin characterization and lignin depolymerization/modification to maximize their values for co-products application.

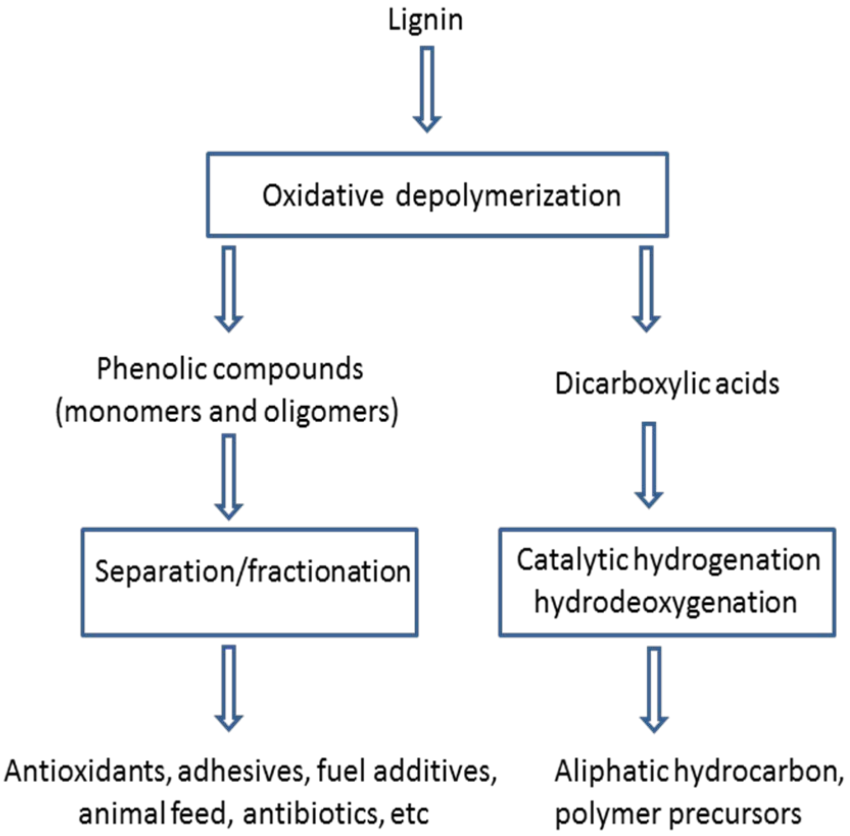


Figure 1. Oxidative conversion of lignin to value added products and fuel precursors.

Activities and Results

Task C-P-2.1 is to assist in optimizing large scale pretreatment and lignin product development.

This task is not scheduled to start until the third year (FY2014) of the project. However, during our recent study, we have found a catalyst that has high selectivity to convert lignin to carboxylic acids. As shown in Figure 2, the softwood lignin was first depolymerized to small molecular phenolic compounds such as vanillic acid and benzoic acid. Increasing reaction time led to the high selectivity toward dicarboxylic acids formation predominantly C4 and C3 acids. We have identified that niobium based catalysts (Cu, Fe, Mn, niobates) can selectively convert lignin to phenolic compounds while a catalyst belongs to a group of sulfide minerals can selectively convert lignin to dicarboxylic acid. The reaction mechanism of the latter reaction is proposed and described in a manuscript under preparation.

Task C-P-2.2 is to study diluted acid pretreatment of Douglas-fir wood and forest residues.

Hemicellulose, which typically accounts for up to 25% of lignocellulosic biomass, is an underutilized component in many biomass conversion processes. A clear understanding of the hemicellulose degradation pathways during different pretreatment conditions can help improve the utilization of hemicellulose. The effect of different diluted acid pretreatment conditions (temperature 180°C-200°C, time 30-90 minutes, acid loading 1-4% gram per gram biomass) on Douglas-fir hemicellulose degradation was first investigated using FS-01 samples. O-acetyl-galactoglucomannan and arabino-4-O-ethylglucuronoxylan are the main constituents of Douglas-fir hemicellulose. As shown in Figure 3, glucose, mannose, xylose, 5-hydroxymethylfurfural (HMF), furfural, levulinic acid (LEV) and formic acid (FA) are the main degradation compounds solubilized during diluted acid pretreatment. The pretreatment time, temperature and acid concentration all have significant effect on softwood hemicellulose degradation. It was found that at 180°C, softwood hemicelluloses were predominantly converted to monosaccharides and their dehydration products, HMF and furfural. However, increasing the pretreatment temperature to 200°C resulted in the further degradation of these compounds to organic acid with a significant amount of levulinic acid and formic acid produced. Controlling the diluted acid pretreatment condition can allow the high yield conversion of hemicellulose to different degradation compounds (Figure 4). For example, maximum concentrations of LEV and FA were detected in the soluble fraction during diluted acid pretreatment of Douglas-fir at 200°C for 90 minutes using 4% (w/w) sulfuric acid which on a weight basis, correlated to approximately 36.0 g of LEV and 17.5 g of FA produced from each 100 g hemicellulose presented in Douglas-fir.

The mass balance of diluted acid pretreatment of FS-01 and FS-03 samples was determined (Figures 5 and 6). Pretreatment was carried out at 195°C using 1% sulfuric acid (w/w) for 30 minutes. As shown in Figure 5, 317.31 kg of glucose can be obtained as monosaccharide from 1000 kg of FS-01. In addition, another 88.94 kg of other sugars (mannose, xylose, galactose) can be obtained. The total sugars available for fermentation is approximately 406 kg. Most of the lignin, after diluted acid pretreatment, is collected in the residues after enzymatic hydrolysis. From 1000kg of FS-01, 256kg of lignin can be obtained as a solid residual after enzymatic hydrolysis. The FS-03 has a lower glucan content and higher lignin content compared to FS-01 (Figure 6). A lower glucose recovery was obtained after dilute acid pretreatment and subsequent enzymatic hydrolysis. From 1000 kg FS-03, 218.3 kg of glucose were obtained as monosaccharide available for fermentation. Also, 73.9 kg of other sugars were recovered in the water soluble fraction. Similar to the FS-01, the recovery yield on lignin in the residual solid is high. Approximately 300 kg of lignin can be obtained from FS-03 after dilute acid pretreatment and subsequent hydrolysis.

Several batch-scale diluted acid pretreatments of Douglas-fir were carried out on FS-03 using 1% H2SO4 for 30 minutes at 195°C. The pretreated solid substrates were hydrolyzed by Novozymes Cellic® CTec-II enzyme preparation. After the hydrolysis, the hydrolysate was separated after centrifugation and solid residues were collected and washed. The washed residues were vacuum dried and approximately 800 grams (o.d) of the resulting residues (DA-HydR) were sent to the co-products team.

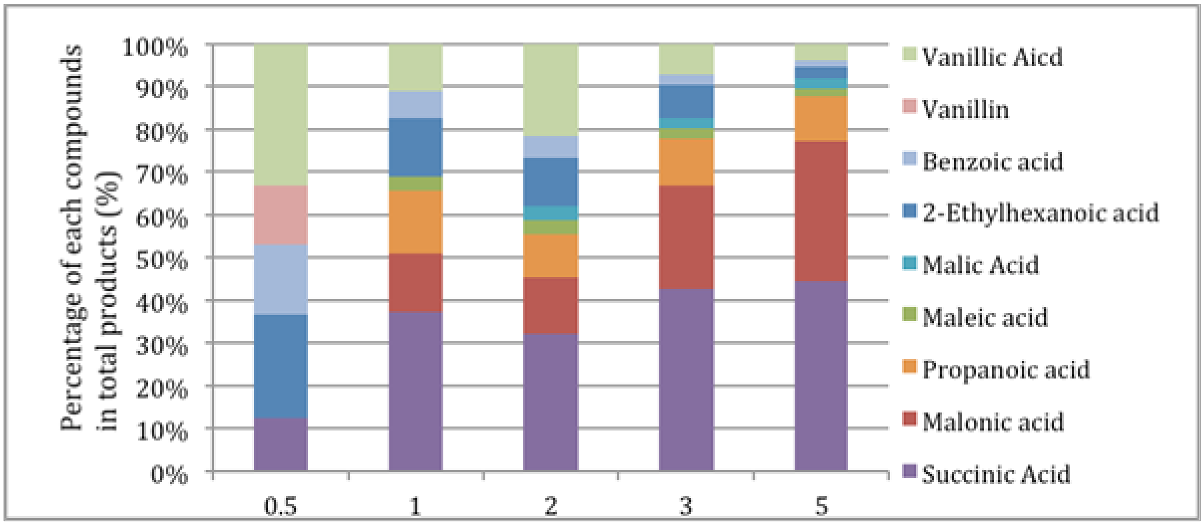


Figure 2: Time dependent reaction products detected during oxidative conversion of softwood lignin (spruce)

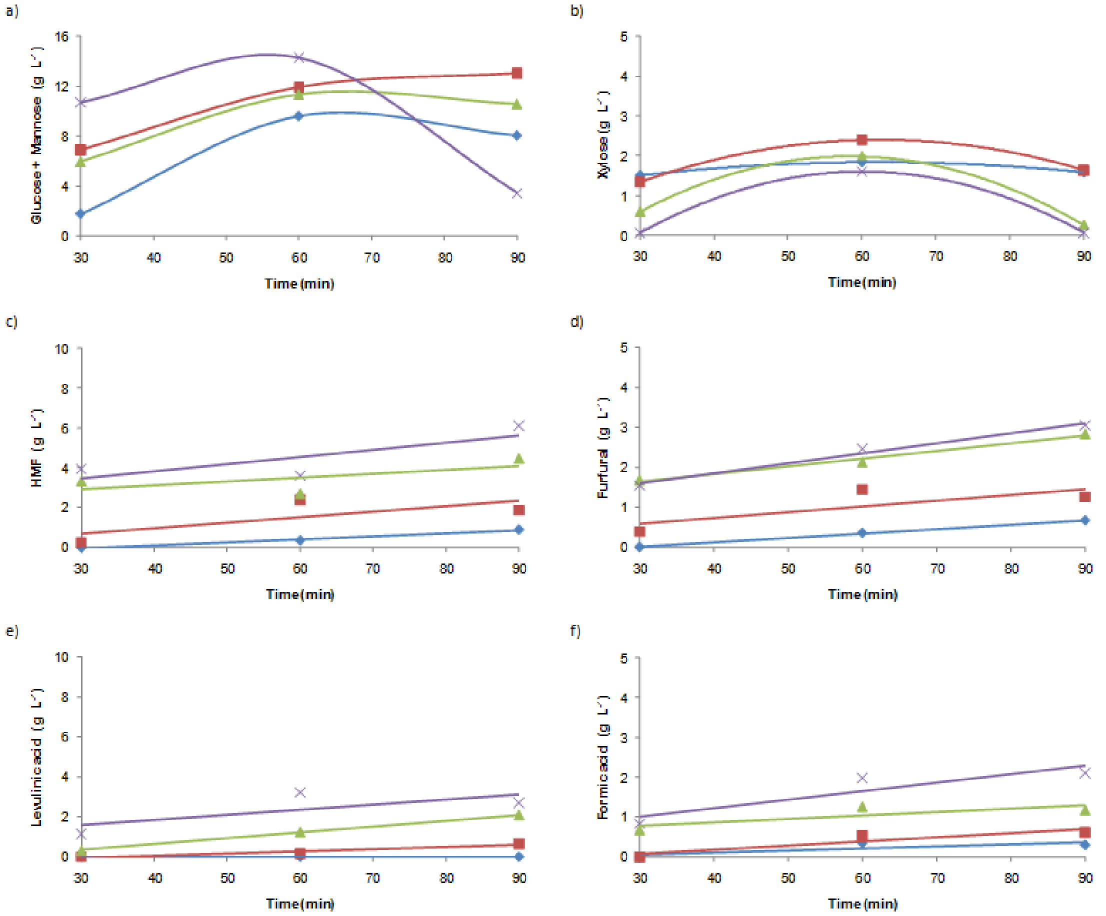


Figure 3: Sugar and decomposition products concentration in WSF during D.A pretreatment of D. fir at180°C: a) Glucose + Mannose, b) Xylose, c) HMF, d) Furfural, e) Levulinic acid, f) Formic acid. Sulfuric acid concentrations: 0% ♦, 1% ■, 2%▲, 4% X.

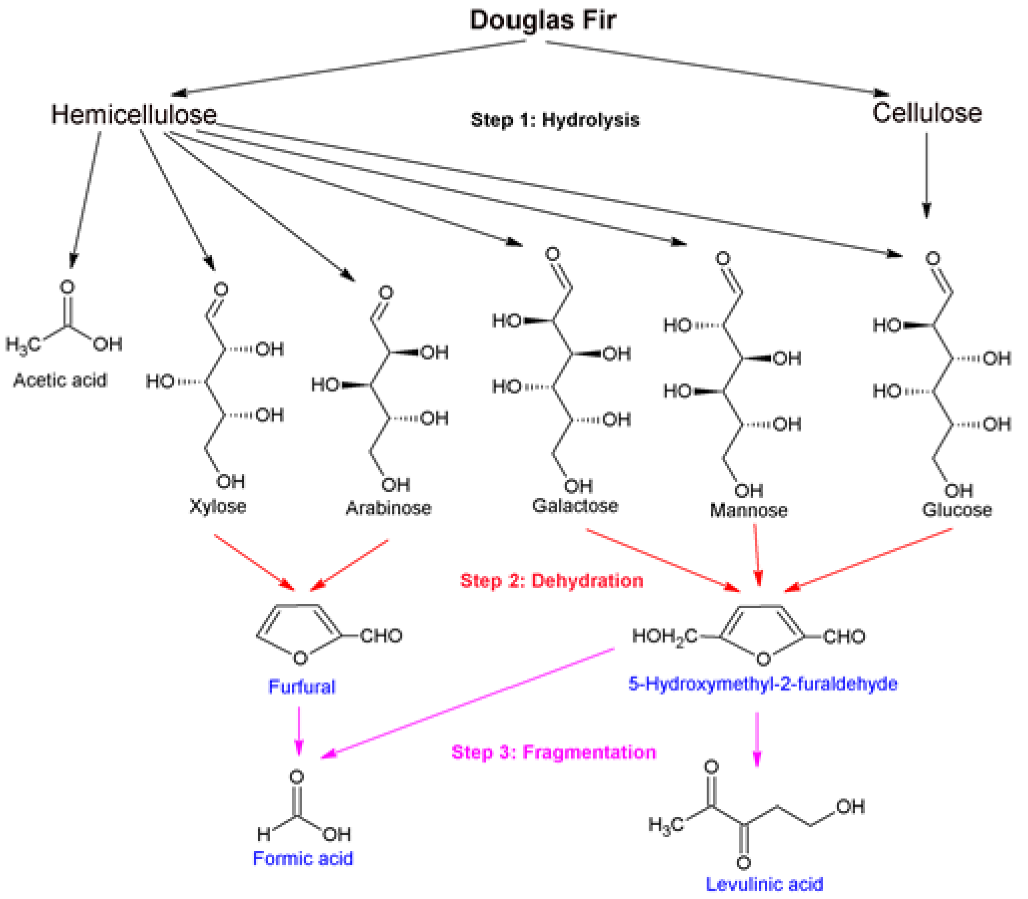


Figure 4: Hemicellulose degradation pathways during diluted acid pretreatment of D. fir.

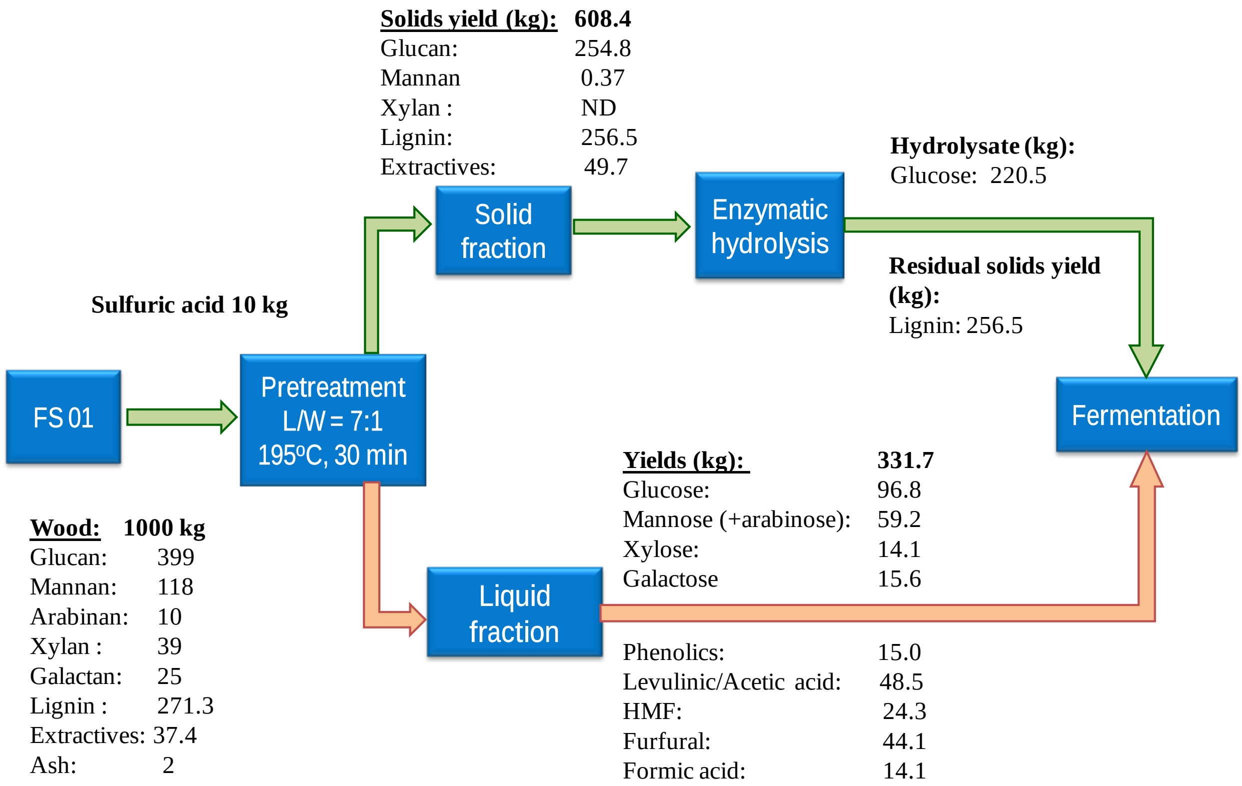


Figure 5: Mass balance of FS01 after diluted acid pretreatment.

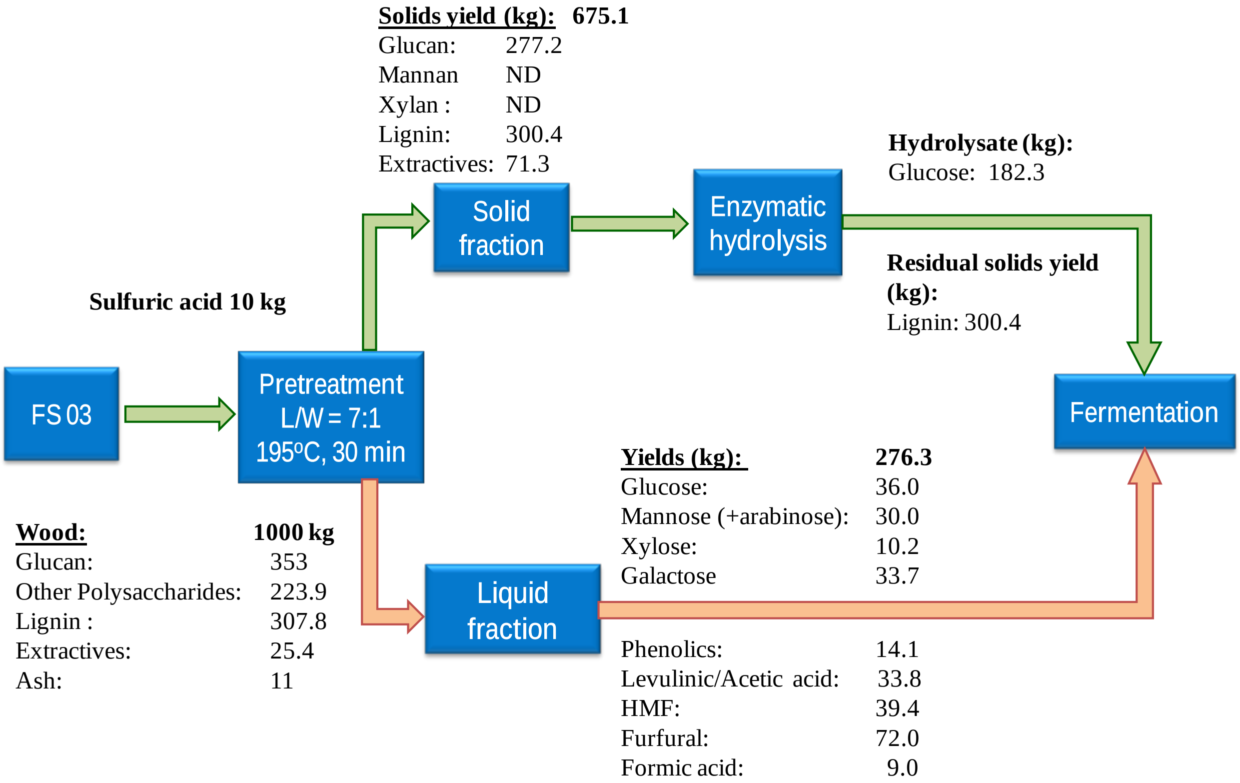


Figure 6: Mass balance of FS03 after diluted acid pretreatment.

Recommendations/Conclusions

Diluted acid presents a relatively simple and low cost pretreatment method and can achieve a high recovery of lignin product with low sulfur and ash. We are investigating diluted acid pretreatment of other Douglas-fir residues and preparing a large batch of dilute acid hydrolyzate from FS-10.

Oxidative degradation of lignin provides a novel and green conversion pathway to produce value added chemicals in high yield. Catalysts with high selectivity to produce either phenolic compounds or dicarboxylic acid were discovered. We plan to start to apply these oxidative chemistries to convert NARA lignin to value added chemicals and polymer precursors.

Physical and Intellectual Outputs

Refereed Publications (accepted or completed)

Alvarez-Vasco, C., and X. Zhang. Elucidate hemicellulose degradation pathways during acid and alkaline pretreatments of softwood: new insight to the production of green chemicals from biomass hemicellulose (submitted)

Conference Proceedings and Abstracts from Professional Meetings

Alvarez-Vasco, C., and X. Zhang. 2012. Understanding the pretreatment chemistry of softwoods. Oral presentation at AICHE Annual Meeting. Pittsburg PA, November 1, 2012.

Research Presentations

Alvarez-Vasco, C., K. Garcia, K. Jayawickrama, E. Brown and X. Zhang. 2012. High throughput analysis of biomass recalcitrance of Douglas fir families. Panel presentation at NARA 2012 Annual Meeting, Missoula, MT, Sept 13-14, 2012.

Alvarez-Vasco, C., M. Guo, E. Brown and X. Zhang. 2012. Diluted acid pretreatment of Douglas fir. Poster presentation at NARA 2012 Annual Meeting, Missoula, MT, Sept 13-14, 2012.

Alvarez-Vasco, C., K. Garcia, K. Jayawickrama, E. Brown and X. Zhang. 2012. Variation in Chemical Composition and Biomass Recalcitrance of D. fir Families. Poster presentation at NARA 2012 Annual Meeting, Missoula, MT, Sept 13-14, 2012.

Pedro Guajardo, Alvarez-Vasco, C, Xiao Zhang “Diluted acid and peroxide pretreatments of Douglas fir biomass” NARA SURE

Intellectual Property

PCT patent application, “METHODS TO CONVERT LIGNIN TO PHENOLIC AND CARBOXYLATE COMPOUNDS” US-842432-01-US-PCT, Xiao Zhang licensed to intellectual ventures

Task C-P-3: Preparation of Pretreated Biomass

Key personnel	Affiliation
Brigitte Ahring	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 the specific task of the project (Task C-P-3.1). The specific operational conditions varied during pretreatment of the different biomass materials included temperature, pressure and oxygen level. The pretreatment process is fully instrumented and allows for full resolution of the optimal pretreatment conditions for the specific woody biomass feedstock. During the year of 2013, the pretreatment team has been strengthened by implementation of a post doc fellow, along with a full time technician. This has allowed us to start making full mass balances over the pretreatment 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 continue to consult with the different NARA 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. Furthermore, the quality of the lignin product produced is an important part of a successful NARA pretreatment process. Conversion results from the fermentation as well as co-product experiments needs to be reported back to WSU BSEL to adjust the pretreatment so that an optimal pretreatment process can be obtained (Task C-P-3.2). In the coming year we propose to optimize coordination to ensure that we will have the fully optimized process developed for both biojet fuel and lignin-products as results of NARA.

Activities and Results

During our preliminary work, we found that pretreatment of Douglas fir was possible at high dry matter content. However, very high dry matter concentrations were not included in the initial pretreatment optimization as equipment related challenges could cause delays. The first part of our study dealt with examining the optimal pretreatment conditions in relation to temperature, time and oxygen loading on the feedstock FS-01. Table 1 summarizes the Wet Explosion Pretreatment conditions tested along with the results obtained. By using the highest initial sugar release, the pretreatment conditions were identified for an enzymatic hydrolysis optimization using dosage response methodology. A summary of the dosage response study is given in Table 2 and Figure 1 using pretreatment conditions of 185C, 25 min, 7.5% oxygen.

Product balances for all the individual process steps were conducted on FS-01 using optimal conditions for initial sugar release after pretreatment and sugar release during enzymatic hydrolysis (conditions for FS-01). Results are shown in Figure 2. The first work on FS-03 showed that the pretreatment conditions would need to be further optimized compared to the results obtained with FS-01. During the optimization period, we were able to increase the sugar yield of FS03 from the original 62% to close to 87% of total sugar released after enzymatic hydrolysis. Unfortunately, the team ran out of FS03 feedstock before the final mass balance could be closed.

Experimental work on FS-10 (replacing FS-03) has begun and will be conducted during second quarter of 2013. From the initial experiments, it is obvious that pretreatment of FS10 will be less challenging when compared to FS03.

Using the optimal pretreatment conditions for FS-01 samples of hydrolysate and residual lignin fractions have distributed to both Gevo and Weyerhaeuser. To ease the work of Gevo samples of up to 50 kg of size are pretreated, enzymatic hydrolyzed in our pilot plant and separated using our screw press and centrifuge yielding a hydrolysate with low concentrations of fibers. The solids containing the lignin residue was frozen along with the clarified hydrolysate and shipped to Weyerhaeuser and Gevo for further work. Two samples of FS-03 have further been shipped to Gevo. The first sample contained higher amounts of inhibitors as the last sample, which was run at more optimal conditions. Lignin samples from these runs have been shipped to Weyerhaeuser for further distribution and testing. The pretreatment team is awaiting feedback from partners to make the necessary adjustments of the conditions so that the samples produced meet the optimal conditions for the biofuels/co-product team.

Table 1. Different Wet Explosion pretreatment conditions and resulting sugar release

Pretreatment Conditions	Cellobiose (g/L)	Glucose (g/L)	Xylose (g/L)	Galactose (g/L)	Arabinose (g/L)	Mannose (g/L)	HMF (g/L)	Furfural (g/L)	Acetate (g/L)
DF-PT-1 185C, 25m, 53 psi O2	1.16	9.55	7.27	5.7	2.18	13.97			
DF-PT-2 185C, 25m, 73 psi O2	1.2	11.53	8.23	6.56	2.52	16.44	2.798	0.522	5.238
DF-PT-3 175C, 25m, 53 psi O2	1.48	5.2	6.87	5.03	2.31	9.81			
DF-PT-4 175C, 25m, 73 psi O2	1.31	6.96	7.39	5.47	2.15	11.74			
DF-PT-5 195C, 25m, 53 psi O2	0.67	9.52	4.07	3.94	1.14	10.98			
DF-PT-6 195C, 25m, 73 psi O2	0.69	11.05	3.97	4.16	1.24	11.13			

Table 2. Summary of sugar release after enzymatic hydrolysis

Table 3. Sugars (g/L)	G+C	Xylose	Galactose	Arabinose	Mannose
C (20mg/g glucan) + H (2mg/g glucan)	70.1	7.7	5.9	2.1	16.9
Cellulase only (20mg/g glucan)	68.8	8.5	7.0	2.0	19.4
Hemicellulase only (2mg/g glucan)	23.4	8.7	6.1	2.2	17.7
C (60mg/g glucan) + H (6mg/g glucan)	121.6	7.6	5.3	1.9	17.6
Cellulase only (60mg/g glucan)	83.8	6.1	2.8	0.7	12.2
Hemicellulase only (6mg/g glucan)	36.1	10.4	8.4	3.7	21.3
C (20mg/g glucan) + H (10mg/g glucan)	82.4	8.0	6.2	1.7	18.2
Cellulase only (20mg/g glucan)	68.8	8.5	7.0	2.0	19.4
Hemicellulase only (10mg/g glucan)	45.5	9.3	7.7	3.4	19.5
C (60mg/g glucan) + H (30mg/g glucan)	142.8	7.1	5.6	1.5	17.5
Cellulase only (60mg/g glucan)	83.8	6.1	2.8	0.7	12.2
Hemicellulase only (30mg/g glucan)	64.7	8.4	5.9	2.8	17.3
C (20mg/g glucan) + H (6mg/g glucan)	79.5	8.1	5.9	1.9	17.9
Cellulase only (20mg/g glucan)	68.8	8.5	7.0	2.0	19.4
Hemicellulase only (6mg/g glucan)	37.8	10.0	8.2	3.5	21.0
C (60mg/g glucan) + H (18mg/g glucan)	134.1	7.7	5.9	1.7	18.3
Cellulase only (60mg/g glucan)	83.8	6.1	2.8	0.7	12.2
Hemicellulase only (18mg/g glucan)	53.3	9.3	8.5	2.6	19.6
C (40mg/g glucan) + H (4mg/g glucan)	99.8	7.7	5.7	1.8	18.1
Cellulase only (40mg/g glucan)	95.7	8.1	5.7	1.7	18.6
Hemicellulase only (4mg/g glucan)	30.9	8.5	5.7	2.6	16.8
C (40mg/g glucan) + H (20mg/g glucan)	106.3	7.5	4.8	1.8	16.4
Cellulase only (40mg/g glucan)	95.7	8.1	5.7	1.7	18.6
Hemicellulase only (20mg/g glucan)	61.4	9.3	7.8	3.4	19.5
C (40mg/g glucan) + H (12mg/g glucan)	103.3	7.9	6.9	1.5	17.3
Cellulase only (40mg/g glucan)	95.7	8.1	5.7	1.7	18.6
Hemicellulase only (12mg/g glucan)	48.1	8.8	6.3	2.1	18.5

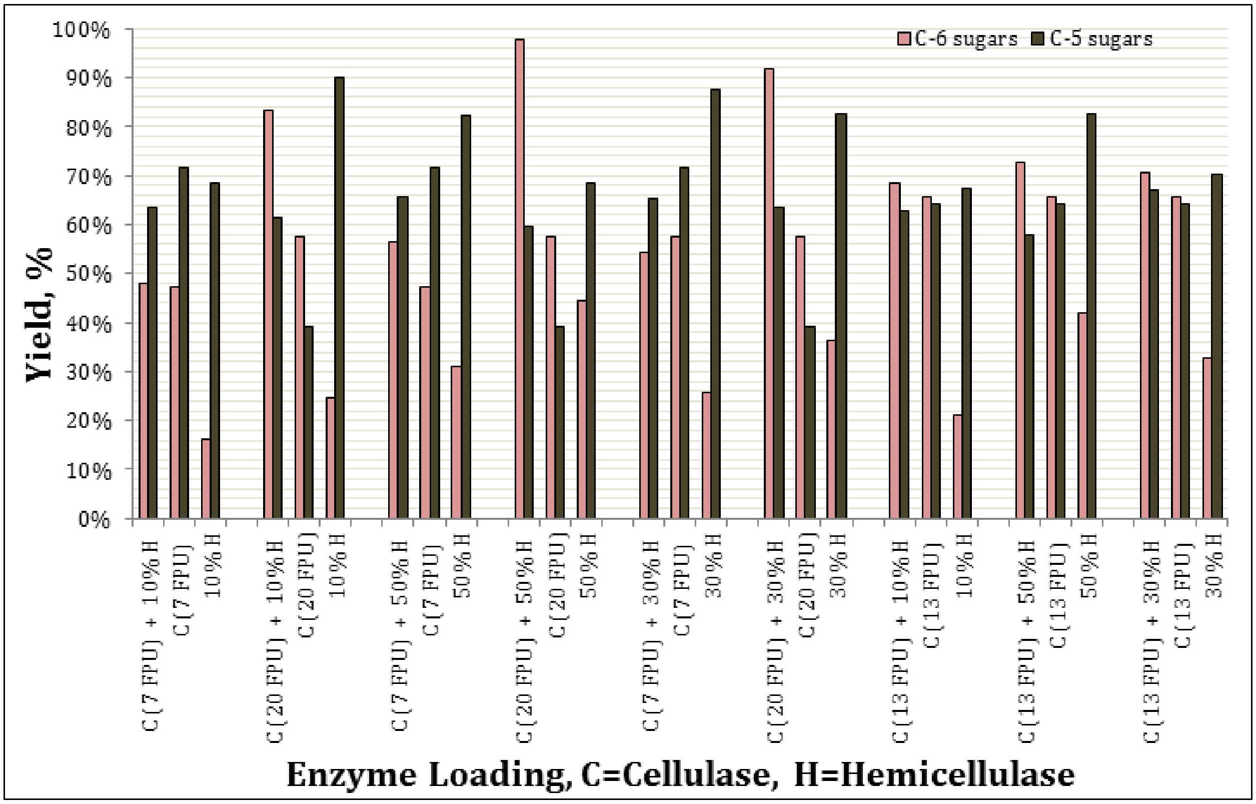
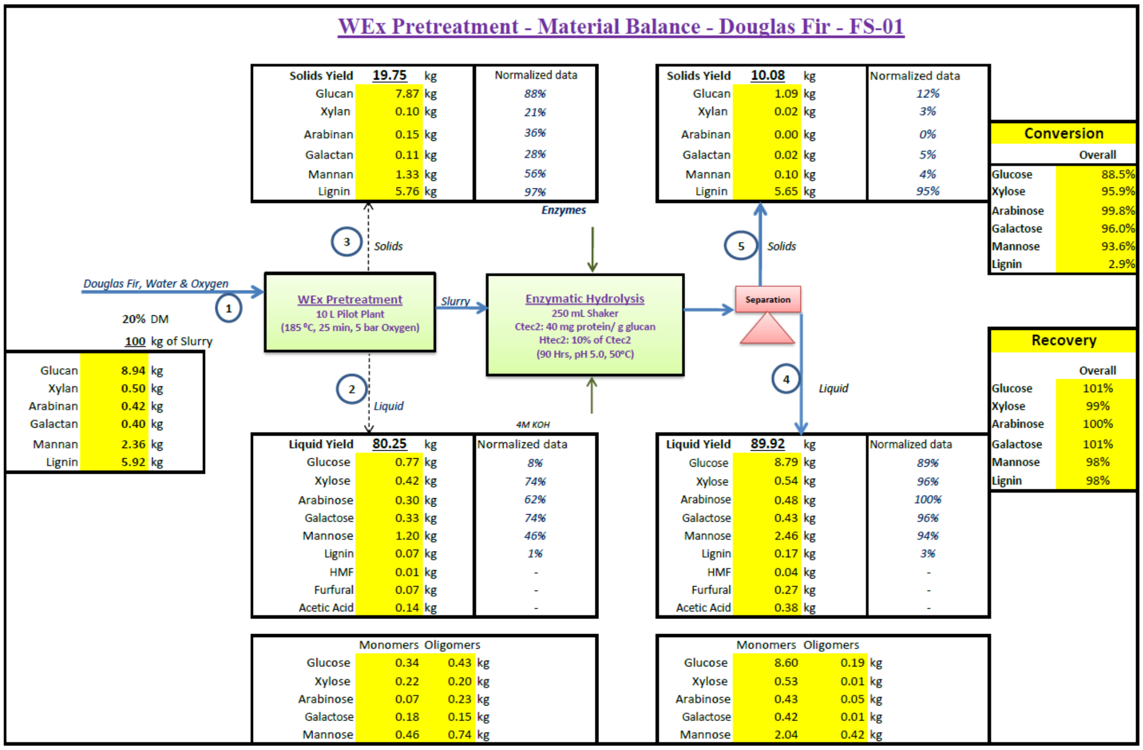


Figure 1. Calculated yield's obtained.



Glucan	8.94	kg
Xylan	0.50	kg
Arabinan	0.42	kg
Galactan	0.40	kg
Mannan	2.36	kg
Lignin	5.92	kg

Solids Yield	19.75	kg	Normalized data
Glucan	7.87	kg	88%
Xylan	0.10	kg	21%
Arabinan	0.15	kg	36%
Galactan	0.11	kg	28%
Mannan	1.33	kg	56%
Lignin	5.76	kg	97%

Solids Yield	10.08	kg	Normalized data
Glucan	1.09	kg	12%
Xylan	0.02	kg	3%
Arabinan	0.00	kg	0%
Galactan	0.02	kg	5%
Mannan	0.10	kg	4%
Lignin	5.65	kg	95%

Conversion	
	Overall
Glucose	88.5%
Xylose	95.9%
Arabinose	99.8%
Galactose	96.0%
Mannose	93.6%
Lignin	2.9%

Recovery	
	Overall
Glucose	101%
Xylose	99%
Arabinose	100%
Galactose	101%
Mannose	98%
Lignin	98%

Liquid Yield	80.25	kg	Normalized data
Glucose	0.77	kg	8%
Xylose	0.42	kg	74%
Arabinose	0.30	kg	62%
Galactose	0.33	kg	74%
Mannose	1.20	kg	46%
Lignin	0.07	kg	1%
HMF	0.01	kg	-
Furfural	0.07	kg	-
Acetic Acid	0.14	kg	-

Liquid Yield	89.92	kg	Normalized data
Glucose	8.79	kg	89%
Xylose	0.54	kg	96%
Arabinose	0.48	kg	100%
Galactose	0.43	kg	96%
Mannose	2.46	kg	94%
Lignin	0.17	kg	3%
HMF	0.04	kg	-
Furfural	0.27	kg	-
Acetic Acid	0.38	kg	-

	Monomers	Oligomers	
Glucose	0.34	0.43	kg
Xylose	0.22	0.20	kg
Arabinose	0.07	0.23	kg
Galactose	0.18	0.15	kg
Mannose	0.46	0.74	kg

	Monomers	Oligomers	
Glucose	8.60	0.19	kg
Xylose	0.53	0.01	kg
Arabinose	0.43	0.05	kg
Galactose	0.42	0.01	kg
Mannose	2.04	0.42	kg

Figure 2: Mass balance – FS-01

Recommendations/Conclusions

The following parameters were found to give the highest digestibility of FS-01 and FS-03:

Pretreatment:

Dry content = 25% (not optimized)

Temp = 185C (optimized)

Time = 25min (optimized)

Oxygen loading = 7.5% of DM (optimized)

Enzyme hydrolysis (for over 95% sugar release from pretreated biomass):

20% DM (not optimized)

40mg EP/Cellulose CTec2 + 10% HTec2 V/V of Ctec2 (optimized)

50°C (optimized)

90h (optimized)

pH=5.0 (optimized)

A major study was done on FS-01 to determine the “sweet spot” where sugar yield and enzyme load is optimized for cost (paper submitted). This study shows that the enzyme load can be decreased to 15 mg EP/ Cellulose CTec2 + 10% HTec2 V/V of Ctec2 with an overall sugar yields of 83%. It is obvious from this study that the cost of sugars is optimized at a yield of sugars that is less than the maximum obtainable. Due to the differences seen in biomass performance during pretreatment, it is recommended that a full optimization study is performed whenever new biomasses are used.Going forward, an optimization study on FS-10 has been planned as well as sample distribution to the partners. Commissioning of the fully equipped Washington State University pilot plant is close and this will allow us to produce much larger samples to meet any needs of the NARA partners.

Physical and Intellectual Outputs

Physical

Table 4: Samples for partners

Gevo	3/14/2012	FS-01-Hydrolysate
Gevo	3/14/2012	FS-01-PT sample
Gevo	12/26/2012	FS-03-Hydrolysate
Gevo	2/18/2013	FS-03-Hydrolysate
Weyerhaeuser	1/21/2013	FS-03 Lignin
Weyerhaeuser	2/18/2013 & 4/16/2013	FS-03 Lignin

Refereed Publications (accepted or completed)

None. However, one paper has been submitted to a peer-reviewed journal summarizing results from FS-01 parameter optimization experiments.

Research Presentations

Ahring, B., D. Rana, V. Rana K. Srinivas and P. Teller. 2012. Breaking the barriers of Douglas fir softwood to biofuels using wet explosion pretreatment. Poster presentation at NARA 2012 Annual Meeting, Missoula, MT, Sept 13-14, 2012.

Task C-P-4: Mild Bisulfite Pretreatment of Forest Residuals

Key Personnel	Affiliation
Dwight Anderson	Catchlight Energy

Task Description

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

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

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

Activities and Results

Mild Bisulfite Pretreatment shows promise to be able to use typically-sized wood chips. This not only reduces the cost associated with more aggressive feedstock processing, but it opens up the possibility of using equipment that is used in existing pulp and paper operations. The task of optimizing Mild Bisulfite Pretreatment began in March 2013. It is focused on the Douglas-fir forest residual reference sample collected from Washington and Oregon in January 2013. This feedstock is a strong candidate for a first commercial plant. The results of the first two pretreatment runs indicate that it is possible to do Mild Bisulfite pretreatment without size reduction of the wood chips other than what is typically practiced in the pulp and paper industry.

A number of parameters in the pretreatment and subsequent processing affect optimization. An experimental plan was developed to begin with Catchlight Energy's current pretreatment parameters and find successively more cost-competitive alternatives. Parameters to be addressed include chip size, chemical concentration, time/temperature, and enzyme optimization.

Chip size is an important characteristic. Many pretreatments require significant feedstock size reduction. This poses two challenges.

- It requires additional capital and energy. Extremely finely-ground wood can require significant energy.

- A high fines content limits equipment selection choices. Wood digesters are mature technology, well-suited to many pretreatment chemistries. The most cost-effective designs cannot tolerate large amounts of fines in the feedstock.

At the same time, however, penetration of wood chips by pretreatment chemistry can be adversely affected by chips that are too large, or by too high a fines content. Excessive fines in a wood chip feedstock consume a disproportionate amount of the pretreatment chemistry compared to typically sized chips in the same feedstock. For these reasons, development work at Catchlight Energy has previously used a reduced chip size with fines removed. This is made by re-chipping wood chips so they pass through a 0.75 inch diameter round hole screen, then discarding the 1/8" fines. These are referred to as the "Reduced-Size Chips" in Table 1. By comparison, the NARA Reference Chips (NARA's FS-10 Combined Accepts) was screened through a 1.75 inch gyratory screen and recombined with overthicks that were milled to <1.5 inches. Fines were not removed, and comprised 9% of the feedstock. Thus, the NARA Reference Chips are less ideal but more practical than the Reduced-Size Chips.

Catchlight Energy has previously found good hydrolysis performance at pulp yields near 75%. The concern was that the more realistic NARA Reference Chips would have too high a yield and too low a chemical consumption compared to the Reduced-Size Chips. Table 1 indicates, however, that the larger chips did not have a higher yield at similar pretreatment conditions, and each cook consumed the same amount of chemical. Thus, future optimization can center on the more practical NARA Reference Chips.

Table 1: Pretreatment Results for Differently-Sized Chips

	Reduced-Size Chips (0.75" screen)	**NARA Reference Chips (1.75" screen)**
Percent Dry Matter	90.56%	88.92%
Ca bisulfite,on wood	11.76%	11.20%
Free SO_2, on wood	4.99%	4.75%
Time to Temperature	45 minutes	55 minutes
Time at Temperature	75 minutes	75 minutes
Temperature	165°C	165°C
Pulp Yield on Wood	68.1%	65.7%
SO_2 consumed during cook	62.1%	61.4%

Recommendations/Conclusions

The Mild Bisulfite pretreatment appears compatible with practical chip sizes that are customarily used in the pulp and paper industry. This will be verified by hydrolyzing the solids to sugar. The next process optimization steps will include decreasing the chemical charge, and altering the time/temperature of the pretreatment.

Physical and Intellectual Outputs

Refereed Publications (accepted or completed)

Prepared independently of NARA funding, but provides background for the starting point of optimization funded by NARA: http://www.biotechnologyforbiofuels.com/content/6/1/10 Gao, Johnway; Anderson, Dwight; Levie, Benjamin; “Saccharification of Recalcitrant Biomass and Integration Options for Lignocellulosic Sugars from Catchlight Energy’s Sugar Process (CLE Sugar),” Biotechnology for Biofuels, **6**:10, Jan. 28, 2013.

Research Presentations

Anderson, D., J. Gao, and B. Levie. 2012. Mild bisulfite pretreatment. Poster presentation at NARA 2012 Annual Meeting, Missoula, MT, Sept 13-14, 2012.

Aviation Team

C-AF-1: Production of Lignocellulosic Isobutanol by Fermentation and Conversion to Biojet

Key Personnel	Affiliation
Andrew Hawkins	Gevo, Inc
Glenn Johnson	Gevo, Inc
Chris Ryan	Gevo, Inc
Bob Wooley	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 1000 gallons (approx. 20 tons feedstock) 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® SSF fermentation from pretreated biomass; (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.

Activities and Results

To address Task C-AF-1.1, analytical data for sugar concentrations and inhibitor concentrations are determined by high pressure liquid chromatography (HPLC) analysis for each feedstock and pretreatment method received. To date, Gevo has received and characterized pretreated materials from NARA feedstock FS-01 and FS-03. SPORL-treated material from Dr. JY Zhu at the USDA Forest Products Laboratory (Madison, WI) and wet oxidation treated material from Dr. Birgitte Ahring at Washington State University – Tricities (Richland, WA) were analyzed from each feedstock. In addition, two hydrolyzates types of pretreated FS-03 material were received from Catchlight Energy and are currently being characterized at Gevo. Concentrations of sugars and inhibitors have been determined for all materials received (Table 1).

Characterization of new hydrolyzates is ongoing and occurs through benchmarking growth and fermentation. Growth performance of a benchmark wild-type ethanol producing strain was compared to an isobutanol producing biocatalyst in different pretreated hydrolyzates using the current NARA feedstock FS-03 in a high-throughput microfermentation system (BioLector, m2p-labs) (Figure 1). Growth and fermentation performance were compared using a wild type ethanol producing strain and the current best isobutanol producing biocatalyst in different hydrolyzates of the current NARA feedstock FS-03. As an example, growth and isobutanol production data from shake flask fermentations using the wet oxidation (WO) adapted strain LB4 is presented in Figure 2.

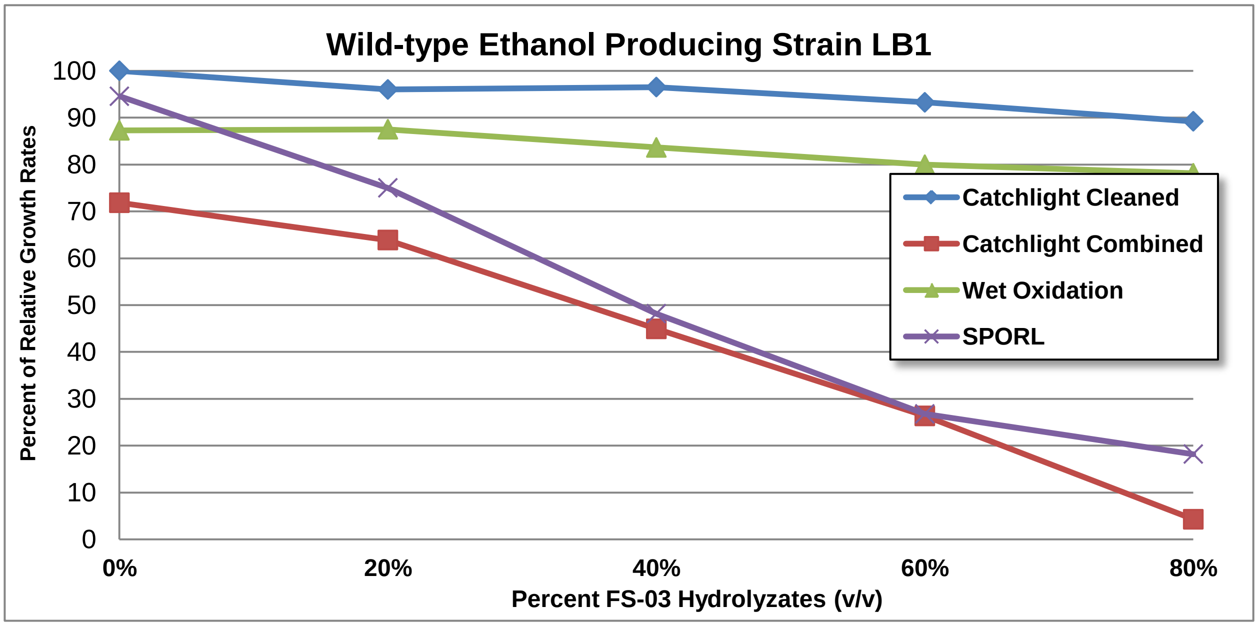
To address task C-AF-1.2, inhibitor concentrations in biomass pretreatments can vary widely depending on the pretreatment method. To generate robust biocatalysts adaptation to a specific pretreated hydrolyzate is needed. A strain adaptation program to generate higher performing isobutanol producing biocatalysts to different hydrolyzates is ongoing. The isobutanol producing biocatalyst LB3 was used as a starting strain for evolutionary engineering using wet oxidation (WO) hydrolyzate. Several improved isolates were identified in a screen for improved growth in WO pretreated biomass derived from FS-01. Comparative growth of one of these adapted strains is presented in Figure 3. The growth of the WO adapted strain was improved over the parental strain for all percentages of WO hydrolyzate. However, the WO adapted strain did not have improved performance in SPORL hydrolyzates above 40% (v/v). Therefore, a similar adaptation program was carried out with the SPORL hydrolyzate using the WO adapted strain LB4 and strains with improved growth in SPORL hydrolyzate were identified and characterized. Figure 4 shows the percent of relative biomass and isobutanol titers for the LB4 parental strain and the SPORL adapted LB17 strain in 50% v/v SPORL black liquor medium. Relative biomass and isobutanol titers were improved for the LB17 strain over the parent LB4. The increased performance of strains adapted to specific hydrolyzates exemplifies the differences in pretreatments and the power of evolutionary engineering. It also indicates the need to focus on a particular pretreatment hydrolysis method, so that a biocatalyst adaptation program may be focused to generate improved performance.

To address Task C-AF-1.3, work began to optimize fermentation conditions for the isobutanol producing biocatalyst strain LB3. This strain is the parent strain of adapted biocatalyst strains such as LB4 and LB17 and serves as a benchmark strain for establishing conditions that can be applied to adapted strains.

To address Task C-AF-1.4, Gevo Process Engineering team members have held several teleconferences and multiple information exchanges with Gevan Marrs (Catchlight Energy) and Tom Spink (TSI, Inc.) to provide information for the NARA techno-economic analysis. Gevo has provided information on its production process unit operations to convert isobutanol to biojet (IPK, isoparafinic kerosene). Gevo has also provided information and direction on how to model the lignocellulosic capital costs using analysis completed by NREL for the production of ethanol and insight on how to adapt this to the production of isobutanol.

Table 1. Sugar and inhibitor concentrations in FS-01 and FS-03 feedstocks from different pretreatments. Compositional analysis was determined using high performance liquid chromatography (HPLC) at Gevo. (n.d. = not detected)

	Glucose (g/L)	**Xylose (g/L)**	**Galactose (g/L)**	**Arabinose (g/L)**	**Mannose (g/L)**	**Acetate (g/L)**	**HMF (g/L)**	**Furfural (g/L)**
FS-01 Wet Oxidation Hydrolyzate	57.20	6.67	5.12	1.58	20.87	7.27	3.90	0.99
FS-03 Wet Oxidation Hydrolyzate	89.58	5.25	3.00	n.d.	7.66	7.00	n.d.	n.d.
FS-01 SPORL Hydrolyzate	93.65	6.89	4.94	1.24	23.01	4.56	0.73	0.07
FS-03 SPORL Hydrolyzate	81.81	5.79	3.82	0.40	7.02	5.78	1.76	0.61
FS-03 Catchlight Combined Hydrolyzate	164.12	8.56	5.24	0.94	13.34	3.45	0.16	0.13
FS-03 Catchlight Cleaned Hydrolyzate	130.89	1.84	0.49	n.d.	1.34	0.26	0.14	0.01



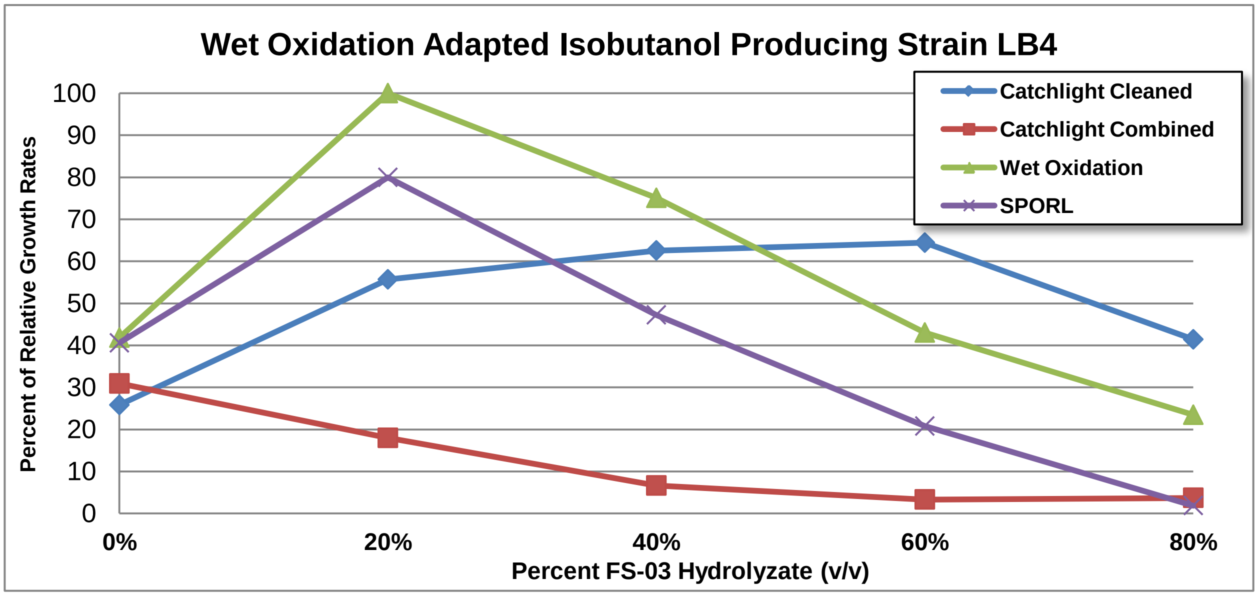


Figure 1. Graphs showing the percent of relative growth rates of the benchmark ethanol producing strain LB1 (Top) and the wet oxidation adapted strain LB4 (Bottom). Data for µMAX values were obtained using a high-throughput microfermentation system (BioLector, m2p-labs). The clarified hydrolyzates preteated using wet oxidation, SPORL, and the process used by Catchlight from NARA feedstock FS-03 were all supplemented with a nutrient package, salts, and a buffering agent. Different percentages of hydrolyzate media contained equal amounts of corresponding sugars and supplements. 100% (v/v) hydrolyzate is equal to approximately 20-30% equivalent solids for all materials.

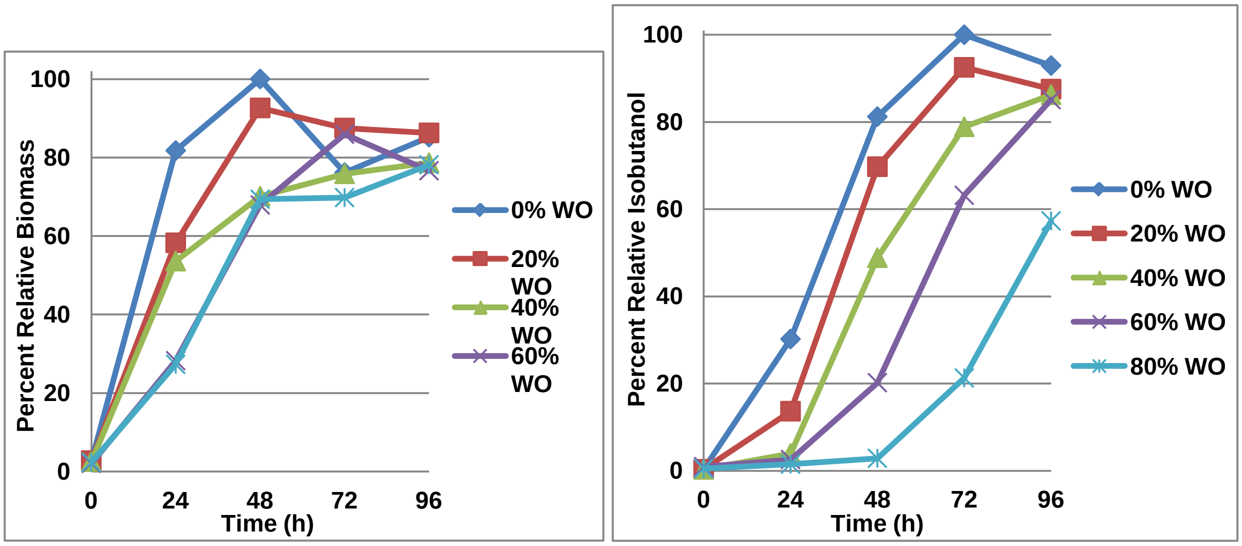


Figure 2. Shake flask fermentations showing the percent relative growth (Left) and relative isobutanol titer (Right) of the wet oxidation adapted parent LB4 in different percentages of FS-03 WO hydrolyzate. The clarified FS-03 wet oxidation pretreated hydrolyzate was supplemented with a nutrient package, salts, and a buffering agent. Different percentages of hydrolyzate media contained equal amounts of corresponding sugars and supplements. At 100% (v/v), not shown, wet oxidation pretreated hydrolyzate was equal to approximately 20-30% equivalent solids. Fermentation was carried out at 33°C for 96 hours in shake flasks. Isobutanol levels were determined by GC analysis.

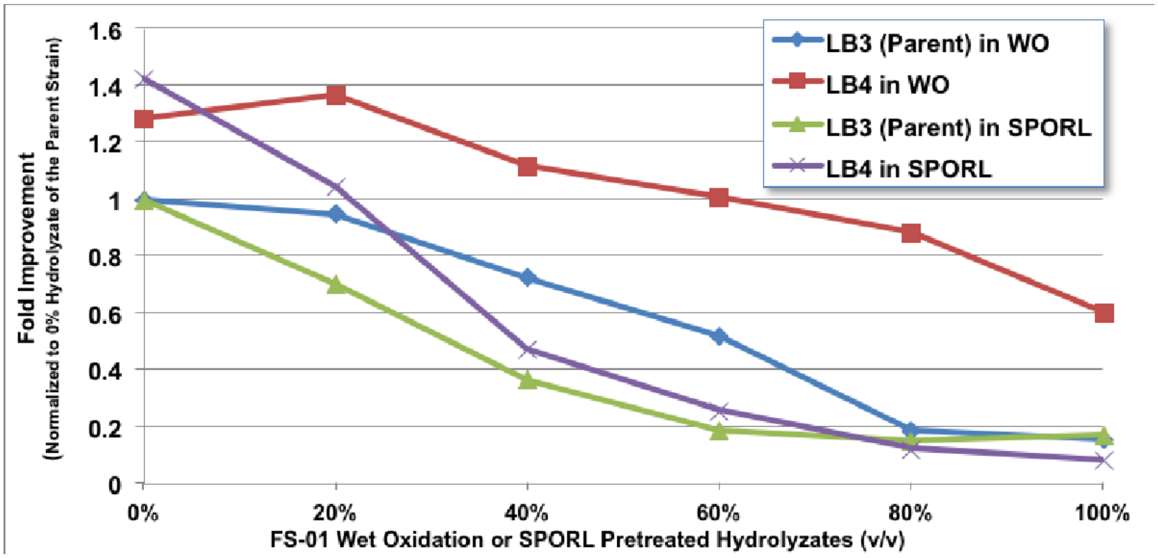


Figure 3. Graph showing the fold improvement in growth rates of the wet oxidation (WO) adapted strain (LB4). Values were normalized to the parental control (LB3) grown in 0% hydrolyzate. Data was obtained using a high-throughput microfermentation system (BioLector, m2p-labs). The clarified FS-01 wet oxidation and SPORL pretreated hydrolyzates were supplemented with a nutrient package, salts, and a buffering agent. Different percentages of hydrolyzate media contained equal amounts of corresponding sugars and supplements. 100% (v/v) SPORL and wet oxidation pretreated hydrolyzate were equal to approximately 20-25% equivalent solids.

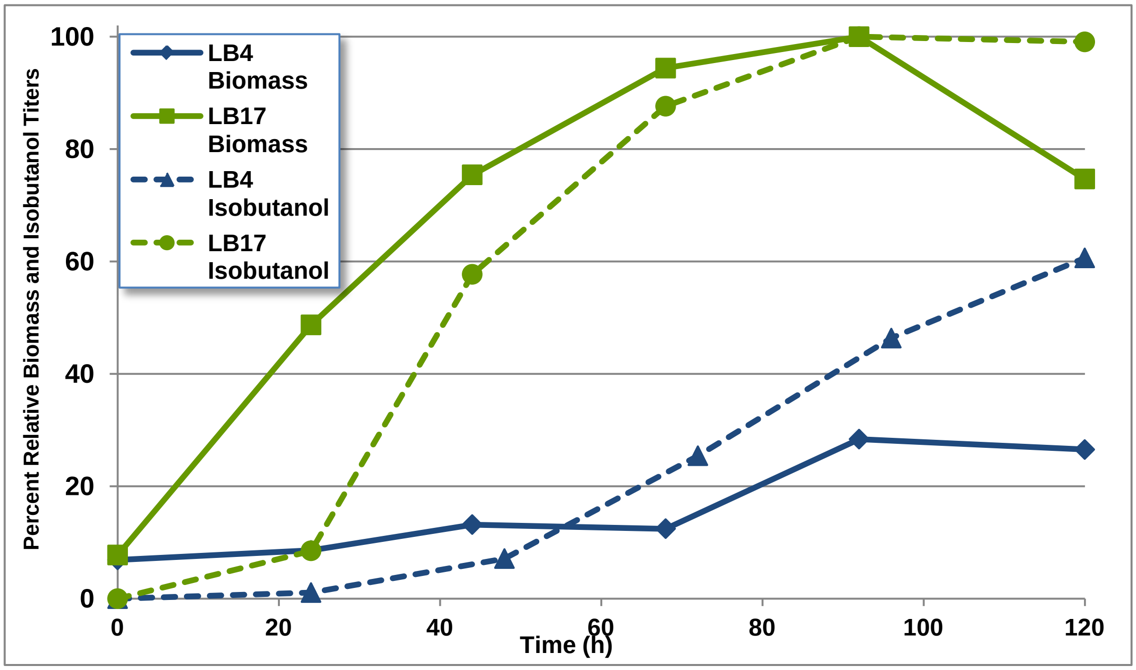


Figure 4. Percent relative growth and isobutanol titers of the wet oxidation adapted parent LB4 compared to a SPORL adapted isolate LB17 both grown in SPORL black liquor (50% v/v) medium derived from SPORL pretreated biomass (FS-01). The liquid black liquor stream was supplemented with a nutrient package, salts, and a buffering agent. Fermentation was carried out at 33°C for 120 hours in shake flasks. Isobutanol levels were determined by GC analysis.

Recommendations/Conclusions

The overall summary to date is that methods for characterizing new hydrolyzate materials have been established. A strain adaptation program to generate higher performing isobutanol producing biocatalysts in different hydrolyzates is currently ongoing. Strains from adaptation programs perform better in the specific hydrolyzate to which they were adapted. In order to obtain the best biocatalyst possible and identify the best conditions for fermentations, narrowing down the types of pretreatments examined will a strain adaptation program to focus on producing improved strains and subsequently determine the optimum parameters for fermentations and scale-up.

Physical and Intellectual Outputs

Physical

None. To date we have not produced physical samples or outputs for transfer to other project components.

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. All results are from pretreated materials that have not been further treated with enzymes. In the project we will concentrate our effort on the following two areas: 1) Co-culture optimization for high production yield and productivity of platform molecules (VFA) from pretreated biomass hydrolysate, and 2) Catalysis of platform molecules into jet fuel. WSU BSEL will work with PNNL (Pacific Northwest National Laboratory) using the PNNL combinatorial catalysis computational laboratory platform.

Activities and Results

Task C-AF-2.1 is to optimize fermentation for making platform molecules. A 40 liter (L) fermentor was setup in the previous quarter and operated on continuous mode. The fermentor is currently producing higher quantities of 2-carbon (C2) and 3-carbon (C3) acids compared to 4-carbon (C4) acids or higher volatile fatty acids (VFA) in the first quarter (total of 32 g/l compared to 22 g/l). The system is fed 10% TS pretreated FS-01. A 5 L lab reactor has been setup to operate in fed-batch mode to form a stable cultured from the 40 L reactor. The sugar and VFA analysis from the first 30-day fed batch reactor run is shown in Figure 1. There is a substantial increase in the production of C3 acid when compared to the continuous fermentor. The system is fed 10% TS pretreated biomass materials and the mass balance shows a very high conversion degree of carbohydrates in the culture (ca. 80%).

In the first quarter of 2013 we concentrated our efforts to produce platform molecules from FS-03. As this material needs a more severe pretreatment compared to FS-01, the amounts of inhibitors are significant higher. The result shown in Fig. 2 clearly demonstrate that the culture is fully capable of handling the new substrate and that stable high yields of especially C2 acid is produced consistently with variable yields of higher acids all the way to C6 acid. The overall yield of acids produced expressed as acetic acid equivalents per gram of total solids or per gram of carbohydrate is shown in Figure 3.

To extract the platform molecules, a pressurized carbon dioxide extraction system has been implemented in our laboratory aimed at the extraction of platform molecules (VFAs) from the fermentation broth(s) (Figure 4). The extraction of acetic acid from water using pressurized carbon dioxide was tested between 25 and 50° C at pressures between 1500 and 2500 psi. A response surface design was set up with concentrations of acetic acid in water varying between 5 g/L and 100 g/L, run time of 1 hour to 5 hours and the carbon dioxide flow rate as measured at the outlet varying between 500 and 1500 mL/min air equivalent (or 350 and 1050 mL/min CO_2 respectively). 32 experiments were run and the experiments were optimized based on both % acetic acid recovery and acetic acid yield (mg acid/g CO_2). It was found that we could extract about 85% of acetic acid from water using carbon dioxide at 50° C, 1500 psi, 1050 mL/min for 5 hours. Statistical analysis of the data indicated that it is possible to get about 98% of the acetic acid extracted from water using carbon dioxide at 2500 psi with all the other experimental conditions remaining the same as described above (Figures 5 and 6).

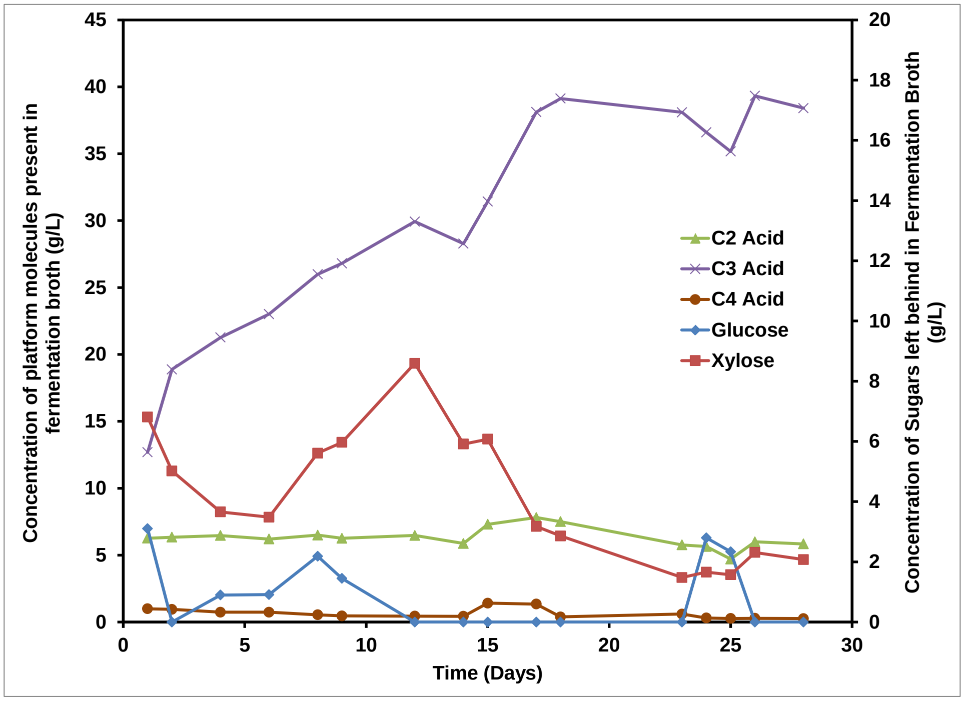
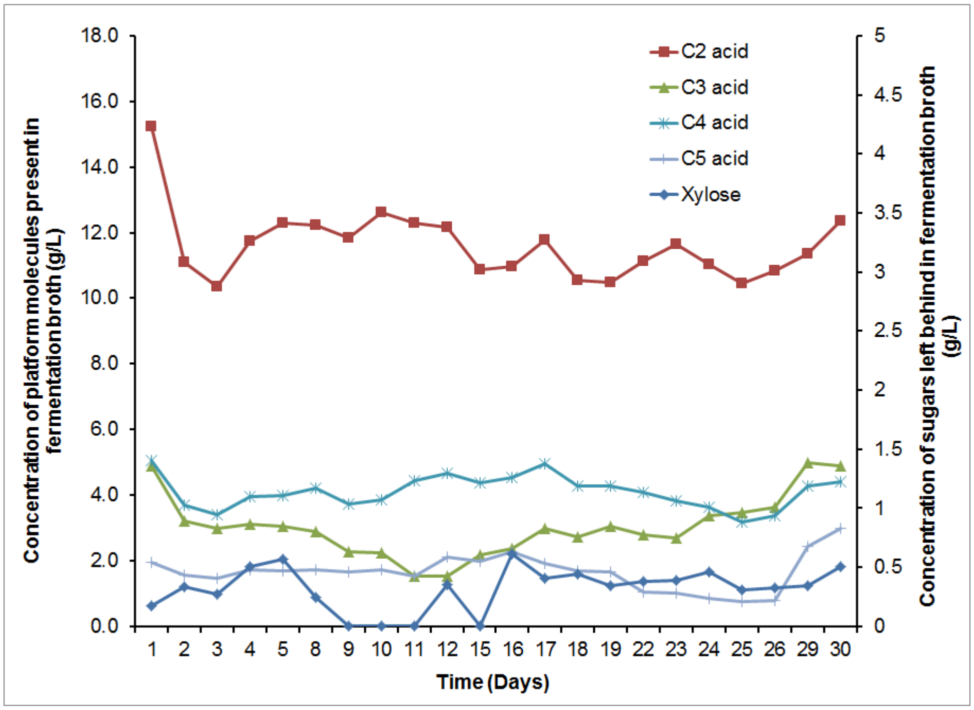


Figure 1: Analysis of Sugars and VFAs in 5L Fermentation Broth Operated in Fed-Batch Mode using pretreated FS01 substrate



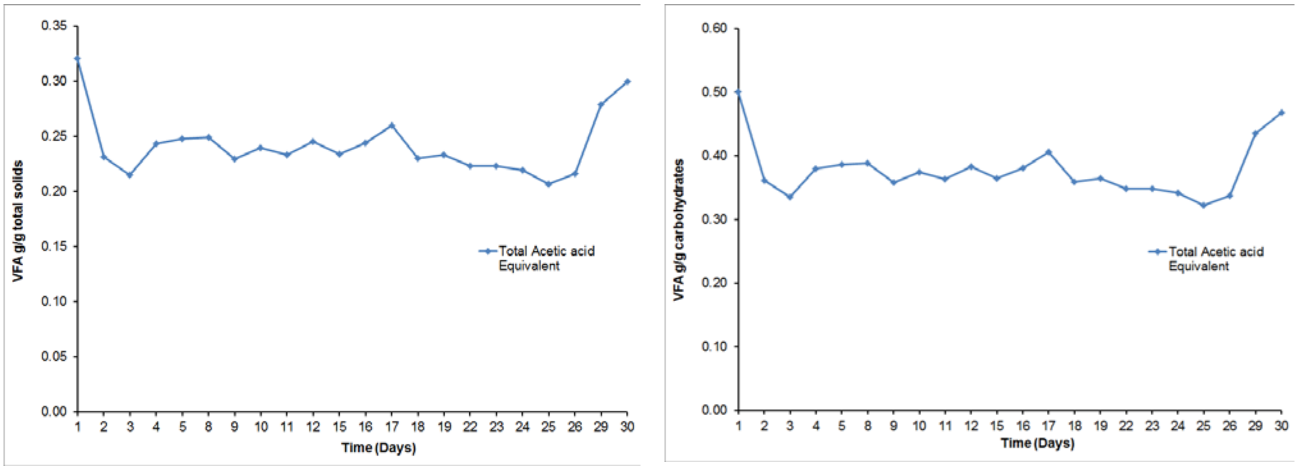


Figure 2: Analysis of Sugars and VFAs in 5L Fermentation Broth Operated in Fed-Batch Mode using pretreated FS03 substrate

Figure 3: Analysis of VFAs as a function of (a) total solids; and (b) sugars in 5 L Fermentation Broth Operated in Continuous Mode with pretreated FS-03 biomass substrate

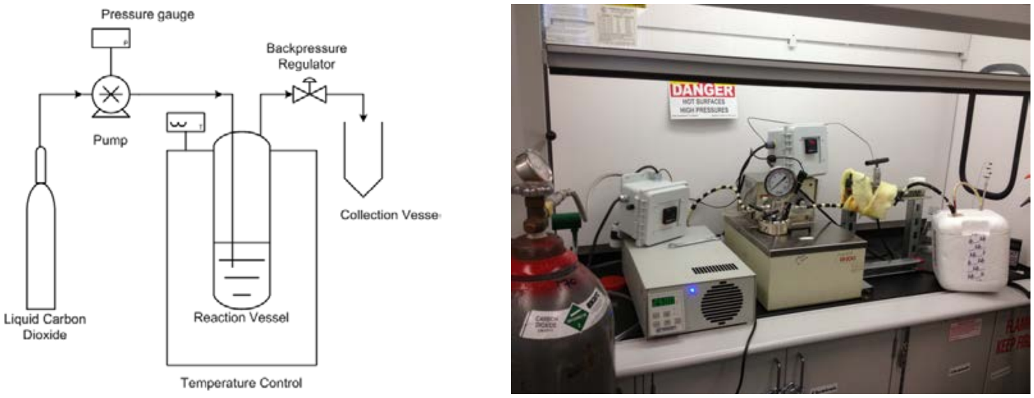


Figure 4: Supercritical carbon dioxide apparatus for extraction of platform molecules

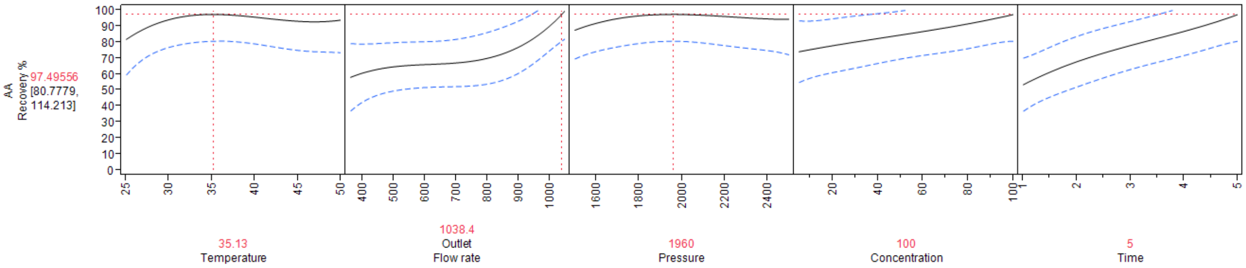


Figure 5: Optimized conditions and maximum acetic acid yield from water using pressurized carbon dioxide – statistical data from response surface experimental design

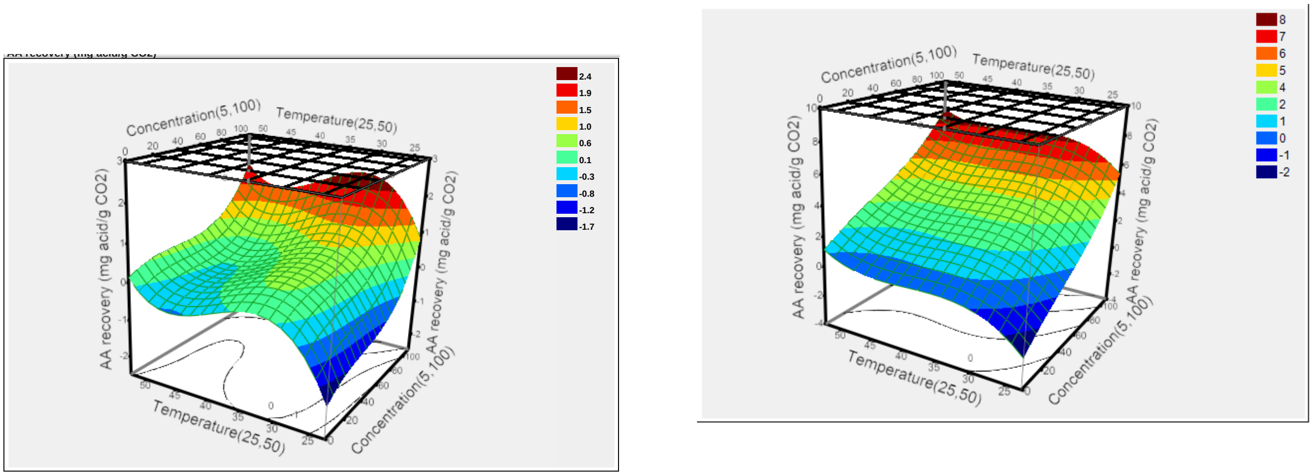


Figure 6: Variation of acetic acid yield (mg acid/g CO_2) as a function of concentration and temperature at 2500 psi and run time of (a) 5 hours; and (b) 1 hour

Recommendations/Conclusions

Stabilization of the culture and increasing the overall productivity has been achieved in the first quarter of 2013. Through optimization of experimental conditions mainly the residence time, the overall yield of C3 acid was found to have increased to as high as 65-70 g/L. Further stabilization is being done of the reactor followed by scale-up to be tested in the second quarter of 2013.

The studies during the period have clearly shown that a fed-batch fermentation of the pretreated woody biomass can provide a stable yield of C2 to C6 acids. Further experiments are being designed to improve the yield from the 5L fed-batch fermenter followed by scale-up under these conditions to a 100L fermenter. Operation of the feed batch reactor with in-situ removal of acids during the fermentation is expected to further improve the fermentation process.

It can be seen from the statistical analysis of the experimental design that the optimized conditions for the extraction of acetic acid from water is 50° C, 2500 psi, outlet CO_2 flow rate of 1050 mL/min and run time of 5 hours. While 5 hours run time was very effective to remove greater amount of acetic acid from water, it was found that greater extraction rate of acetic acid happened in shorter run times (1 hr or less). For example, it was found that under the afore-mentioned conditions, the total acetic acid extracted at the end of 5 hours at 1500 psi was found to be around 3 mg acid/g CO_2 (Figure 6a) while the amount extracted at the end of the first hour was found to be around 7 mg acid/g CO_2 (Figure 6b). The reason for this difference is because at higher flow rates as a function of pressure, greater extraction happens in the first hour or so followed by continuous extraction at a slower rate as run time is increased. Further experiments are currently being done to study the kinetics of pressurized carbon dioxide extraction of acetic acid from water and fermentation broth.

It can be seen from Figure 5 that temperature does not have too significant an effect on the extraction efficiency between 35° and 50° C. It can also be seen from Figure 5 that the percent recovery of acetic acid using pressurized carbon dioxide varied linearly with the concentration of acetic acid in the feed. This indicates that the higher the amount of acetic acid in the feed, the higher the extraction efficiency of carbon dioxide will be. This can be attributed to the non-polar nature of acetic acid that makes it more attractive to pressurized carbon dioxide when compared to water. This behavior of the volatile fatty acids and the non-polarity of pressurized carbon dioxide as a solvent make this separation process more attractive to extract these platform molecules directly from the fermentation broth. It is also evident from the experiments that the headspace has a significant effect on the extraction rate as it would increase the residence time and hence, decrease the total run time to get the same acid yield. Further experiments will be designed to increase the headspace in the reactor through recirculation of the pressurized carbon dioxide to increase both the percent recovery of acetic acid as well as the extraction rate, thereby significantly reducing the total run time of the extraction.

Physical and Intellectual Outputs

None. The process is still being optimized and hence, no physical outputs have been obtained yet.

Refereed Publications (accepted or completed)

None. However, two manuscripts are under preparation and are planned to be submitted to journals in the start of the next quarter.

Conference Proceedings and Abstracts from Professional Meetings

Research Presentations

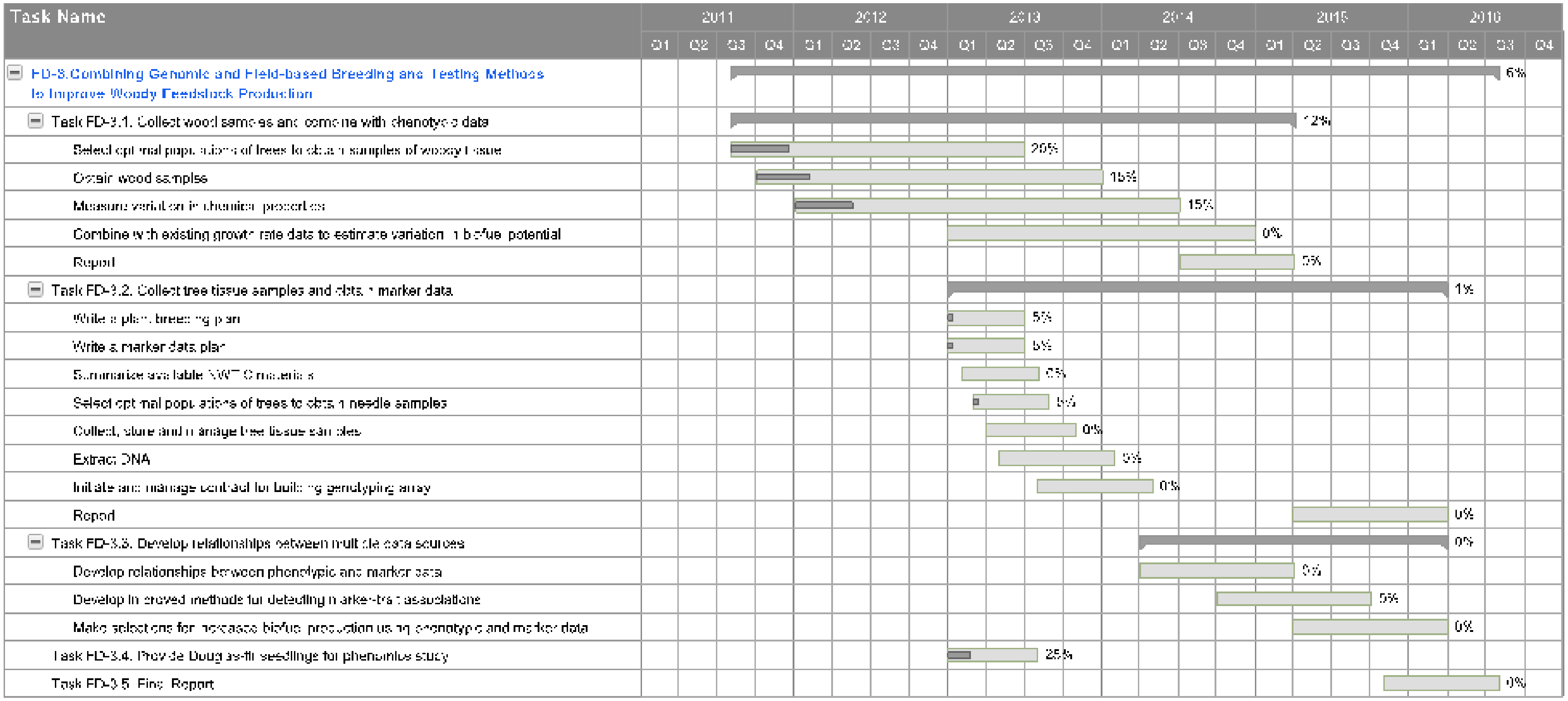
Ahring, B.K., D. Rana, V. Rana, and P. Teller. 2012. Producing high sugar yields from softwood using wet explosion pretreatment. 34th SBFC Meeting, New Orleans, LA. Apr 30-May 2, 2012.

Ahring, B.K., D. Rana, P.Teller. 2012. Wet explosion pretreatment of different feedstocks and its potential for producing biofuels. Pacific Rim Summit on Industrial Biotechnology and Bioenergy, Vancouver. Oct 10-12.

Ahring, B.K. 2012. New development for production of hydrocarbon biofuels. Frontier in Biorefining. St. Simon Island, Georgia. Oct 30-Nov 2.

Ahring, B.K. 2012. Panel Meeting and Presentation at NARA 2012 Annual Meeting, Missoula, MT, Sept 13-14, 2012.

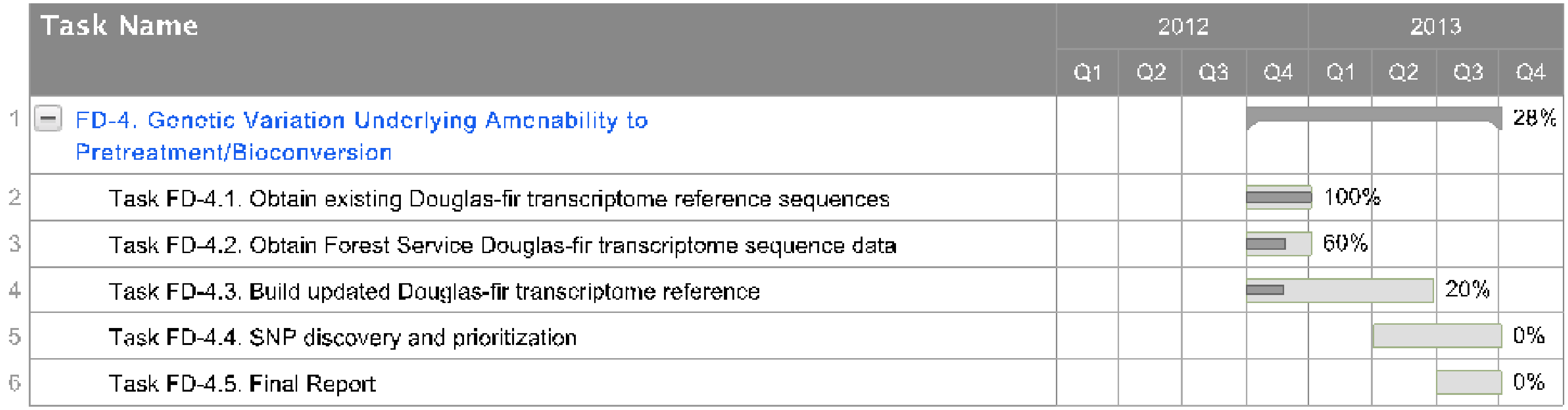
FeedstockDevelopment_Jayawickrama



	Task Name	
1	FD-3.Combining Genomic and Field-based Breeding and Testing Methods to Improve Woody Feedstock Production	6%
2	Task FD-3.1. Collect wood samples and combine with phenotype data	12%
3	Select optimal populations of trees to obtain samples of woody tissue	20%
4	Obtain wood samples	15%
5	Measure variation in chemical properties	15%
6	Combine with existing growth rate data to estimate variation in biofuel potential	0%
7	Report	5%
8	Task FD-3.2. Collect tree tissue samples and obtain marker data	1%
9	Write a plant breeding plan	5%
10	Write a marker data plan	5%
11	Summarize available NWTC materials	0%
12	Select optimal populations of trees to obtain needle samples	5%
13	Collect, store and manage tree tissue samples	0%
14	Extract DNA	5%
15	Initiate and manage contract for building genotyping array	0%
16	Report	0%
17	Task FD-3.3. Develop relationships between multiple data sources	0%
18	Develop relationships between phenotypic and marker data	0%
19	Develop improved methods for detecting marker-trait associations	5%
20	Make selections for increased biofuel production using phenotypic and marker data	0%
21	Task FD-3.4. Provide Doug-fir seedlings for phenomics study	25%
22	Task FD-3.5. Final Report	0%

FeedstockDevelopment_NCGR

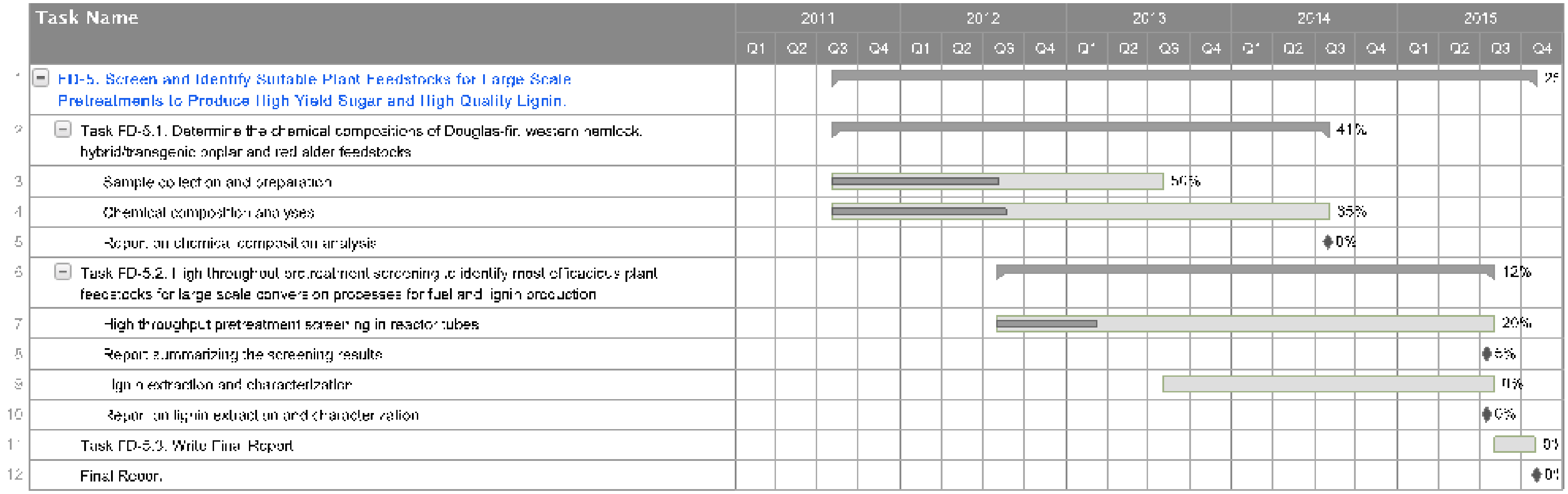
NARA



	Task Name	2012 Q1	2012 Q2	2012 Q3	2012 Q4	2013 Q1	2013 Q2	2013 Q3	2013 Q4
1	FD-4. Genetic Variation Underlying Amenability to Pretreatment/Bioconversion								28%
2	Task FD-4.1. Obtain existing Douglas-fir transcriptome reference sequences					100%			
3	Task FD-4.2. Obtain Forest Service Douglas-fir transcriptome sequence data					60%			
4	Task FD-4.3. Build updated Douglas-fir transcriptome reference							20%	
5	Task FD-4.4. SNP discovery and prioritization								0%
6	Task FD-4.5. Final Report								0%

FeedstockDevelopment_XZhang

NARA



FeedstockLogistics_Marrs

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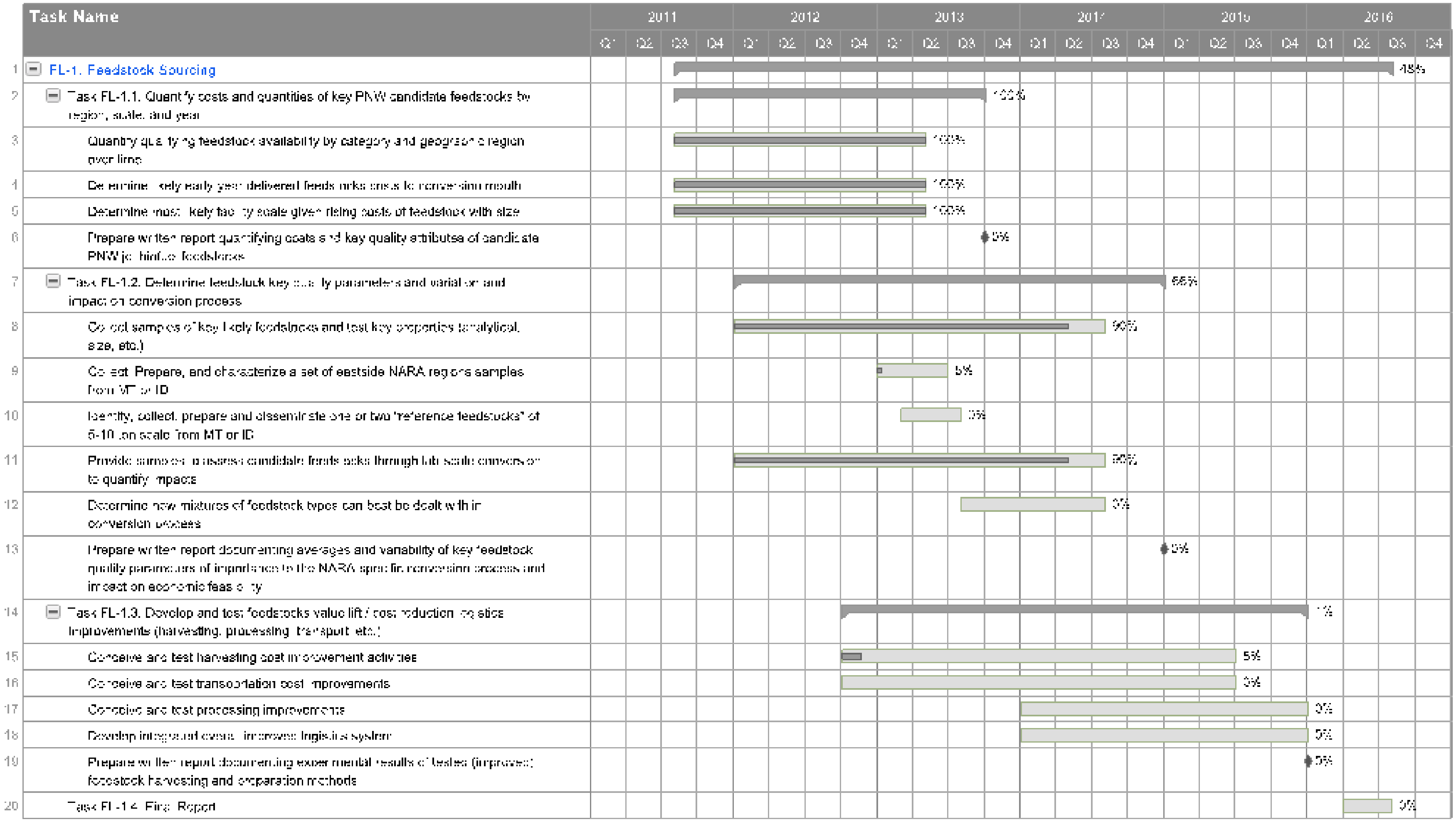
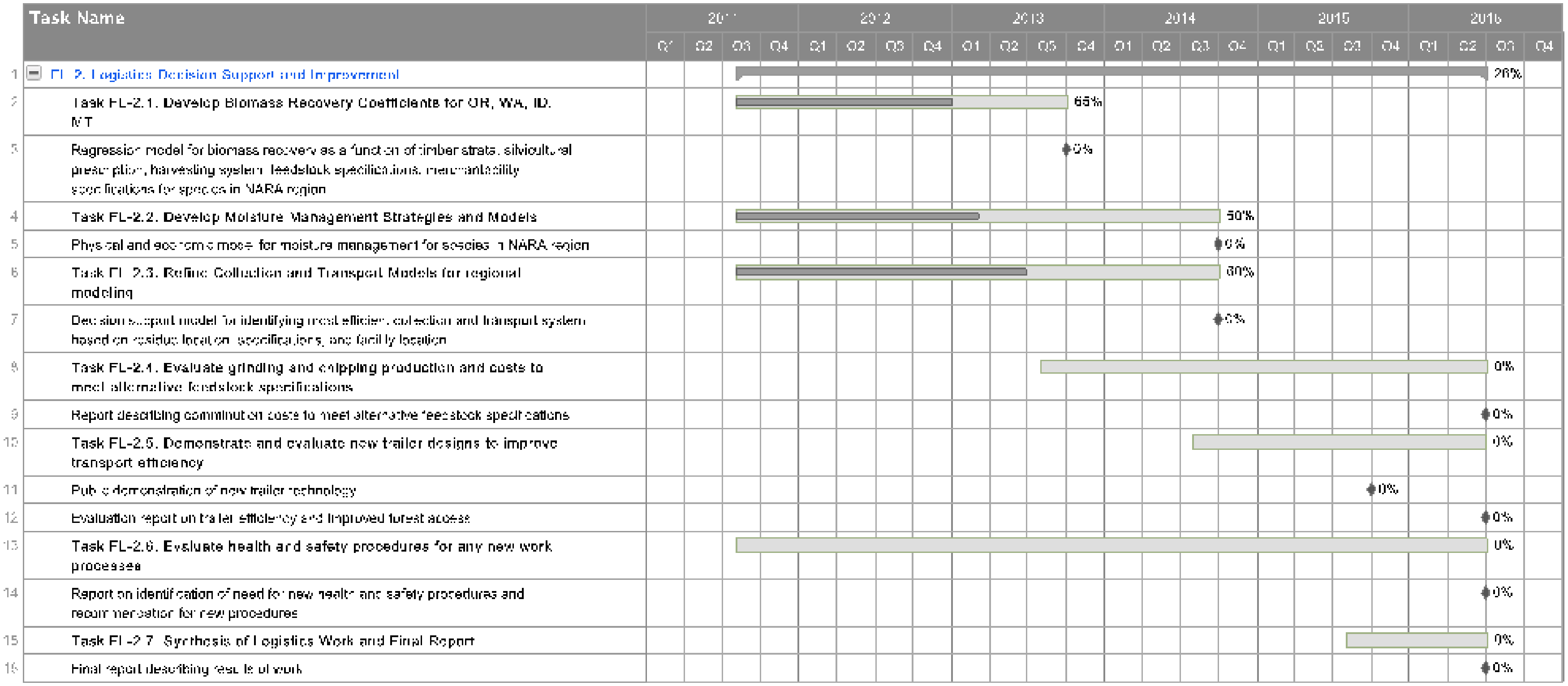


	Task Name
1	FL-1. Feedstock Sourcing
2	Task FL-1.1. Quantify costs and quantities of key PNW candidate feedstocks by region, scale, and year
3	Quantify qualifying feedstock availability by category and geographic region over time
4	Determine likely early year delivered feedstocks costs to conversion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

FeedstockLogistics_Sessions

NARA



	Task Name	
1	FL-2. Logistics Decision Support and Improvement	26%
2	Task FL-2.1. Develop Biomass Recovery Coefficients for OR, WA, ID, MT	65%
3	Regression model for biomass recovery as a function of timber strata, silvicultural prescription, harvesting system, feedstock specifications, merchantability specifications for species in NARA region	0%
4	Task FL-2.2. Develop Moisture Management Strategies and Models	50%
5	Physical and economic model for moisture management for species in NARA region	0%
6	Task FL-2.3. Refine Collection and Transport Models for regional modeling	60%
7	Decision support model for identifying most efficient collection and transport system based on residue location, specifications, and facility location	0%
8	Task FL-2.4. Evaluate grinding and chipping production and costs to meet alternative feedstock specifications	0%
9	Report describing comminution costs to meet alternative feedstock specifications	0%
10	Task FL-2.5. Demonstrate and evaluate new trailer designs to improve transport efficiency	0%
11	Public demonstration of new trailer technology	0%
12	Evaluation report on trailer efficiency and improved forest access	0%
13	Task FL-2.6. Evaluate health and safety procedures for any new work processes	0%
14	Report on identification of need for new health and safety procedures and recommendation for new procedures	0%
15	Task FL-2.7. Synthesis of Logistics Work and Final Report	0%
16	Final report describing results of work	0%

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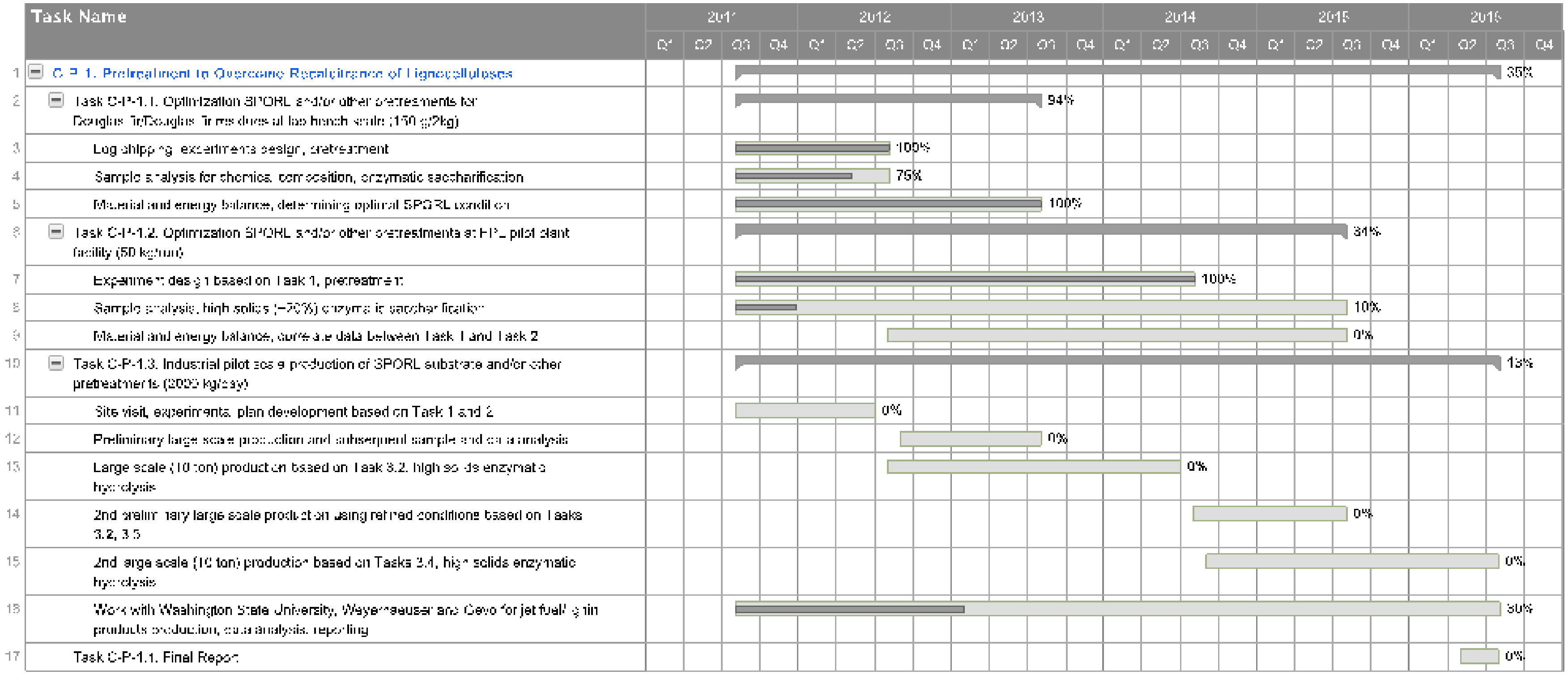
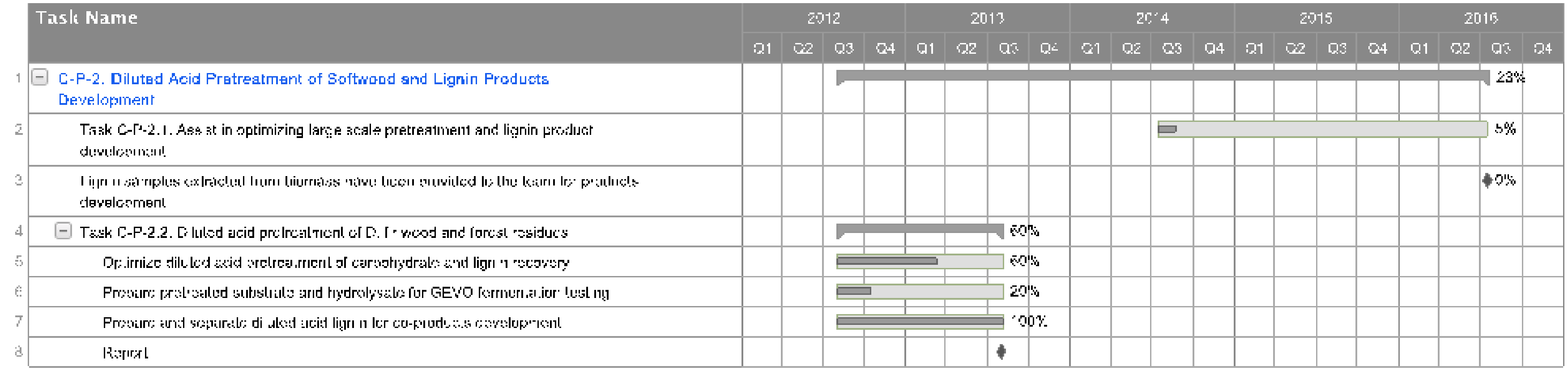
Pretreatment_Zhu



	Task Name	
1	C-P-1. Pretreatment to Overcome Recalcitrance of Lignocelluloses	35%
2	Task C-P-1.1. Optimization SPORL and/or other pretreatments for Douglas fir/Douglas fir residues at lab bench scale (150 g/2kg)	94%
3	Log shipping, experiments design, pretreatment	100%
4	Sample analysis for chemical composition, enzymatic saccharification	75%
5	Material and energy balance, determining optimal SPORL condition	100%
6	Task C-P-1.2. Optimization SPORL and/or other pretreatments at FPL pilot plant facility (50 kg/run)	34%
7	Experiment design based on Task 1, pretreatment	100%
8	Sample analysis, high solids (~20%) enzymatic saccharification	10%
9	Material and energy balance, correlate data between Task 1 and Task 2	0%
10	Task C-P-1.3. Industrial pilot scale production of SPORL substrate and/or other pretreatments (2000 kg/day)	13%
11	Site visit, experiments, plan development based on Task 1 and 2	0%
12	Preliminary large scale production and subsequent sample and data analysis	0%
13	Large scale (10 ton) production based on Task 3.2, high solids enzymatic hydrolysis	0%
14	2nd preliminary large scale production using refined conditions based on Tasks 3.2, 3.5	0%
15	2nd large scale (10 ton) production based on Tasks 3.4, high solids enzymatic hydrolysis	0%
16	Work with Washington State University, Weyerhaeuser and Gevo for jet fuel/lignin products production, data analysis, reporting	30%
17	Task C-P-1.1. Final Report	0%

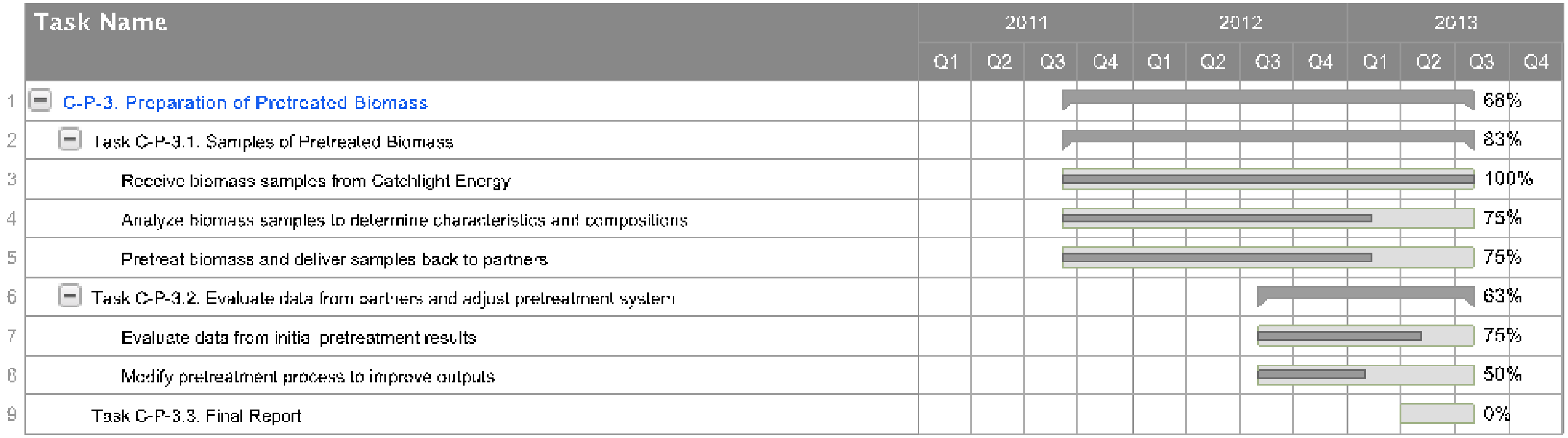
Pretreatment_XZhang



	Task Name	
1	⊟ C-P-2. Diluted Acid Pretreatment of Softwood and Lignin Products Development	23%
2	Task C-P-2.1. Assist in optimizing large scale pretreatment and lignin product development	5%
3	Lignin samples extracted from biomass have been provided to the team for products development	0%
4	⊟ Task C-P-2.2. Diluted acid pretreatment of D. Fir wood and forest residues	60%
5	Optimize diluted acid pretreatment of carbohydrate and lignin recovery	60%
6	Prepare pretreated substrate and hydrolysate for GEVO fermentation testing	20%
7	Prepare and separate diluted acid lignin for co-products development	100%
8	Report	

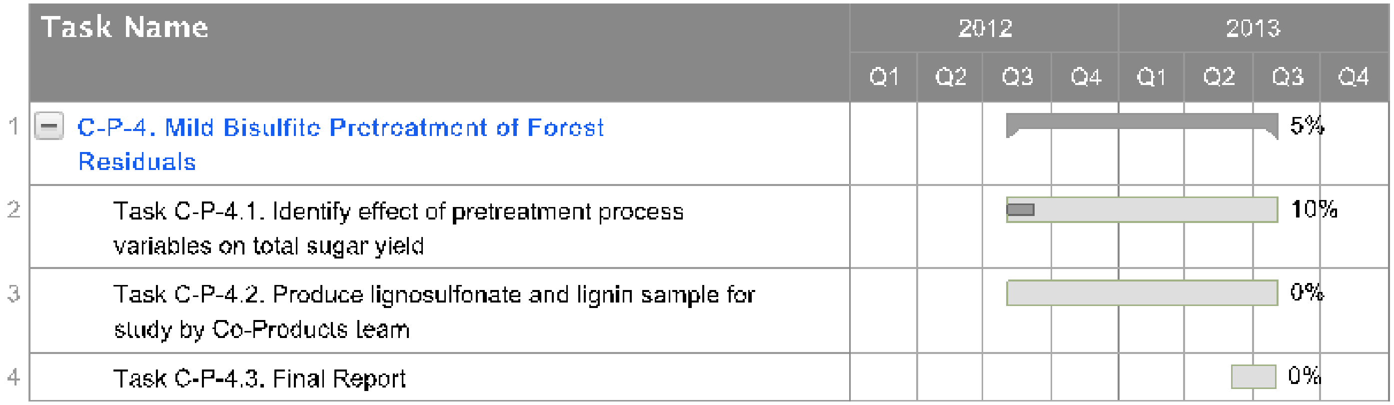
Pretreatment_Ahring

NARA



	Task Name	
1	⊟ C-P-3. Preparation of Pretreated Biomass	68%
2	⊟ Task C-P-3.1. Samples of Pretreated Biomass	83%
3	Receive biomass samples from Catchlight Energy	100%
4	Analyze biomass samples to determine characteristics and compositions	75%
5	Pretreat biomass and deliver samples back to partners	75%
6	⊟ Task C-P-3.2. Evaluate data from partners and adjust pretreatment system	63%
7	Evaluate data from initia pretreatment results	75%
8	Modify pretreatment process to improve outputs	50%
9	Task C-P-3.3. Final Report	0%

Pretreatment_Catchlight



1 C-P-4. Mild Bisulfite Pretreatment of Forest Residuals

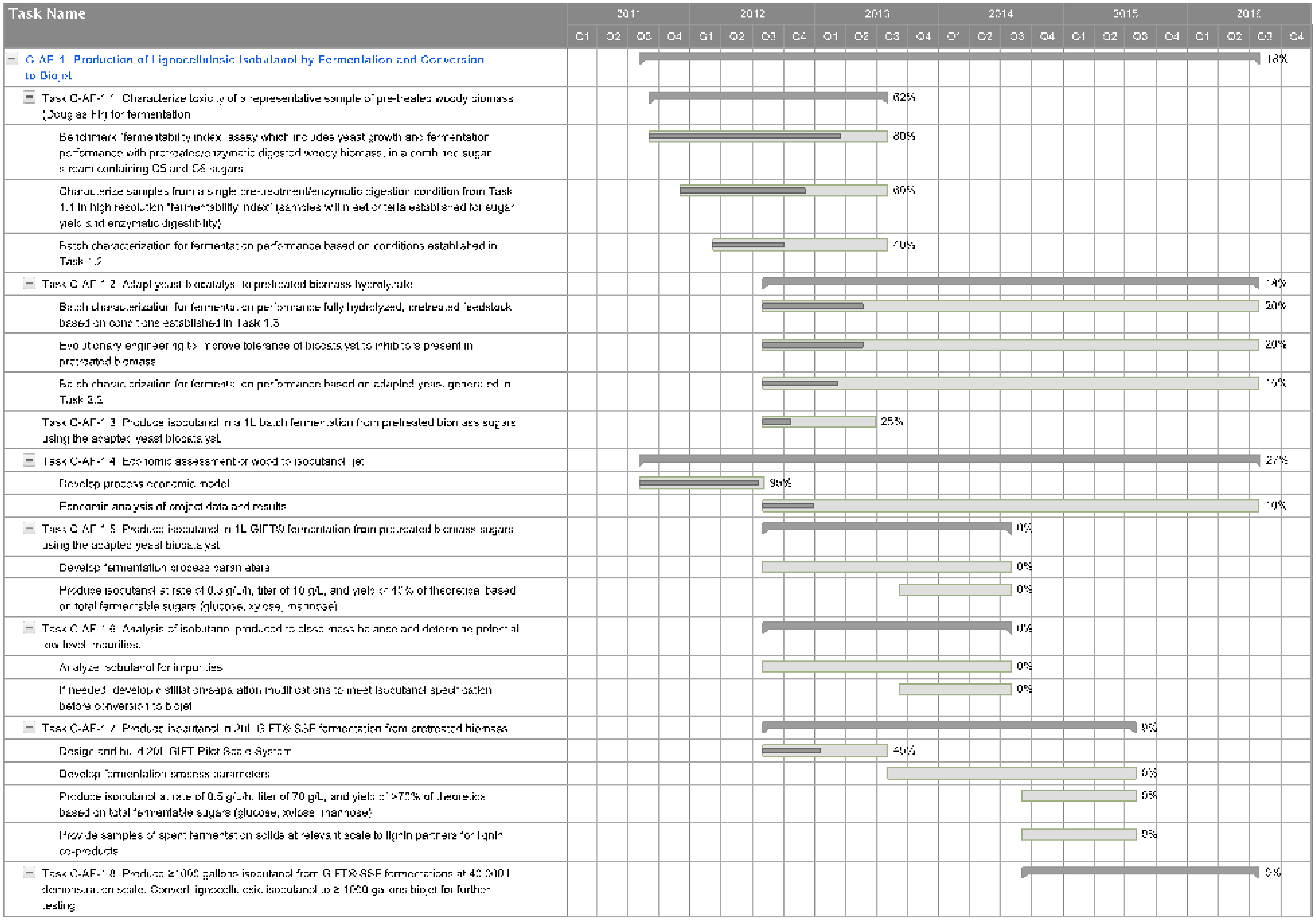
2 Task C-P-4.1. Identify effect of pretreatment process variables on total sugar yield

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

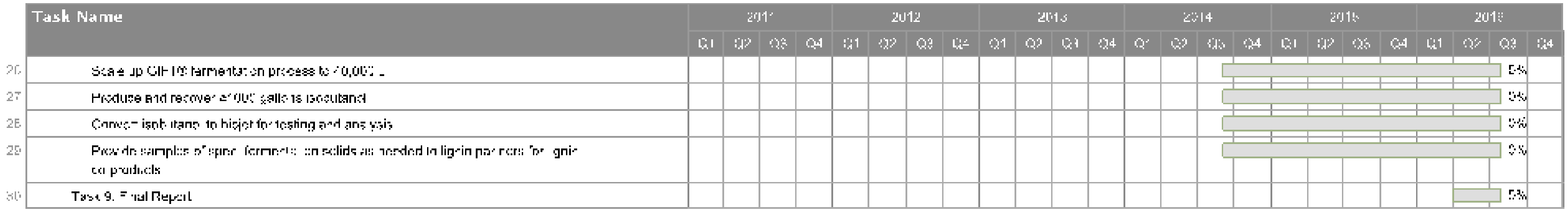
4 Task C-P-4.3. Final Report

Conversion-AF_Gevo

NARA



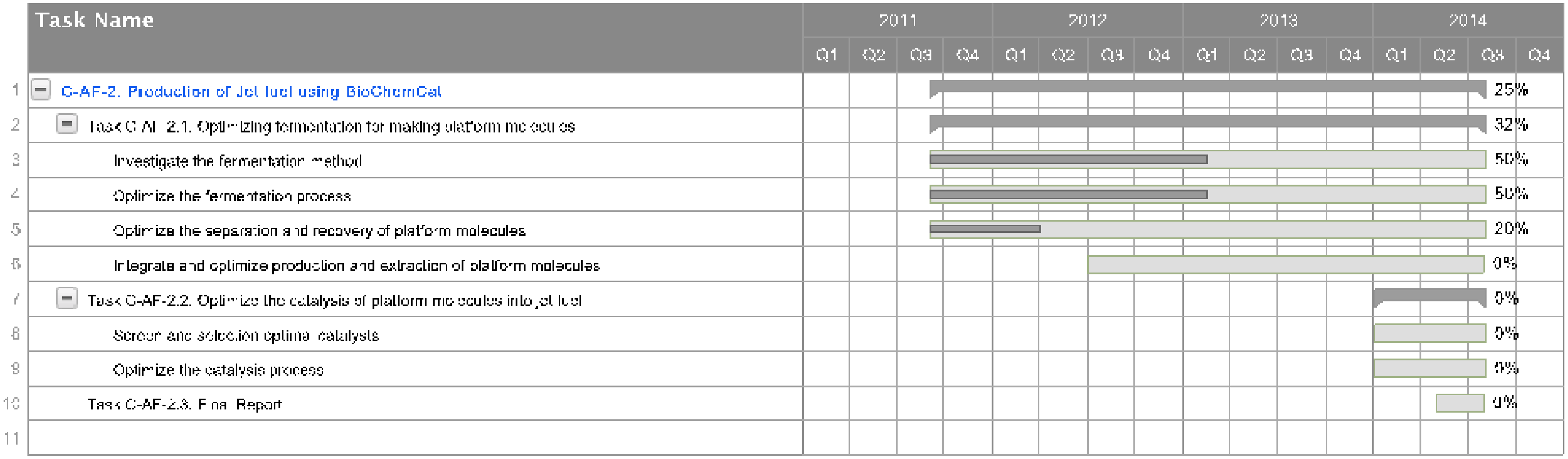
	Task Name	
1	C-AF-1: Production of Lignocellulosic Isobutanol by Fermentation and Conversion to Biojet	18%
2	Task C-AF-1.1: Characterize toxicity of a representative sample of pretreated woody biomass (Douglas Fir) for fermentation	62%
3	Benchmark "fermentability index" assay which includes yeast growth and fermentation performance with pretreated/enzymatic digested woody biomass, in a combined sugar stream containing C5 and C6 sugars	80%
4	Characterize samples from a single pre-treatment/enzymatic digestion condition from Task 1.1 in high resolution "fermentability index" (samples will meet criteria established for sugar yields and enzymatic digestibility)	60%
5	Batch characterization for fermentation performance based on conditions established in Task 1.2	70%
6	Task C-AF-1.2: Adapt yeast biocatalyst to pretreated biomass hydrolysate	14%
7	Batch characterization for fermentation performance fully hydrolyzed, pretreated feedstock based on conditions established in Task 1.3	20%
8	Evolutionary engineering to improve tolerance of biocatalyst to inhibitors present in pretreated biomass	20%
9	Batch characterization for fermentation performance based on adapted yeast generated in Task 2.2	10%
10	Task C-AF-1.3: Produce isobutanol in a 1L batch fermentation from pretreated biomass sugars using the adapted yeast biocatalyst	25%
11	Task C-AF-1.4: Economic assessment of wood to isobutanol to jet	27%
12	Develop process economic model	90%
13	Economic analysis of project data and results	10%
14	Task C-AF-1.5: Produce isobutanol in 1L GIFT® fermentation from pretreated biomass sugars using the adapted yeast biocatalyst	0%
15	Develop fermentation process parameters	0%
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)	0%
17	Task C-AF-1.6: Analysis of isobutanol produced to close mass balance and determine potential trace level impurities	0%
18	Analyze isobutanol for impurities	0%
19	If needed, develop distillation/separation modifications to meet isobutanol specification before conversion to biojet	0%
20	Task C-AF-1.7: Produce isobutanol in 20L GIFT® SSF fermentation from pretreated biomass	9%
21	Design and build 20L GIFT Pilot Scale System	40%
22	Develop fermentation process parameters	0%
23	Produce isobutanol at rate of 0.5 g/L/h, titer of 70 g/L, and yield of >75% of theoretical based on total fermentable sugars (glucose, xylose, mannose)	0%
24	Provide samples of spent fermentation solids at relevant scale to lignin partners for lignin co-products	0%
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	0%



	Task Name	%
26	Scale up GIFT® fermentation process to 20,000 L	0%
27	Produce and recover 1000 gallons isobutanol	0%
28	Convert isobutanol to biojet for testing and analysis	0%
29	Provide samples of spent fermentation solids as needed to lignin partners for lignin co-products	0%
30	Task 9. Final Report	0%

Conversion-AF_Ahring

NARA



	Task Name	
1	C-AF-2. Production of Jet fuel using BioChemCat	25%
2	Task C AF 2.1. Optimizing fermentation for making platform molecules	32%
3	Investigate the fermentation method	50%
4	Optimize the fermentation process	50%
5	Optimize the separation and recovery of platform molecules	20%
6	Integrate and optimize production and extraction of platform molecules	0%
7	Task C-AF-2.2. Optimize the catalysis of platform molecules into jet fuel	0%
8	Screen and selection optimal catalysts	0%
9	Optimize the catalysis process	0%
10	Task C-AF-2.3. Final Report	0%
11		