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Douglas-fir core samples used to determine recalcitrance variability.

Screening Douglas-fir Trees for Production of Biofuels and Other Valuable Chemicals

Douglas-fir trees are not all alike. Some grow faster, some adapt better to harsher climates and they all differ somewhat in their chemical makeup. These distinctions are due to variations in their genetic makeup. Those interested in breeding plantation trees for timber value have capitalized on genetic variation to select softwoods with characteristics (traits) that provide better timber products and reduce production costs.

NARA was established to facilitate development of a new industry that uses the residual wood in slash piles to produce chemical products like biojet fuel. As this industry develops, softwood breeders will be interested in bringing seedlings to the marketplace that not only enhance timber production but also allow improved chemical conversion from the residuals.

Measuring Carbohydrates, Lignin and Recalcitrance

Forest residuals that provide high carbohydrate yields at low cost would be an ideal feedstock for biojet fuel production. NARA researchers in the Feedstock Development Team wanted to know if carbohydrate (simple sugars) yields varied among Douglas-fir samples. If variation did exist, then the genetic causes for the variation could potentially be identified and used to breed softwood trees that produced superior residuals for conversion to biojet fuel and other chemical products.

To test carbohydrate yield variation among Douglas-fir trees, a research team lead by Xiao Zhang and Keith Jayawickrama sampled 150 plantation-grown Douglas-fir trees, representing 40 Douglas-fir families, for variation in carbohydrate yield and published their results in the journal Bioenergy Research.

> View *A Multi-Level Analysis Approach to Measuring Variations in Biomass recalcitrance of Douglas Fir Tree Samples*

Carbohydrate yields are determined by carbohydrate content and, more importantly, by the level of recalcitrance. Recalcitrance, in this case, refers to the resistance wood has to deconstruction. A high level of recalcitrance would inhibit carbohydrate extraction more than a lower level. For this study, the authors developed a high throughput screening process to determine the chemical composition and level of recalcitrance for each sample.

Douglas-fir Trees Exhibit Variation in Chemical Composition and Recalcitrance

There was a large degree of chemical variation evident among the samples tested. Extractives (fatty acids, resin acids, waxes and terpenes) ranged from 0.25 to 5.1% of the wood composition. Lignin ranged from 23 to 39.9%, and total polysaccharides ranged from 62.1 to 78.8%.

To measure the level of recalcitrance, two steps were required. The first step involved subjecting the samples to pretreatment, used to remove the lignin and hemicelluloses from the carbohydrates stored as cellulose. Enzymatic hydroly-

sis represented the second step, where enzymes were added to the pretreated material to liberate the individual simple sugars from the cellulose. The simple sugars released were measured; the simple sugar yield compared to the total amount of carbohydrates available represented a level of recalcitrance.

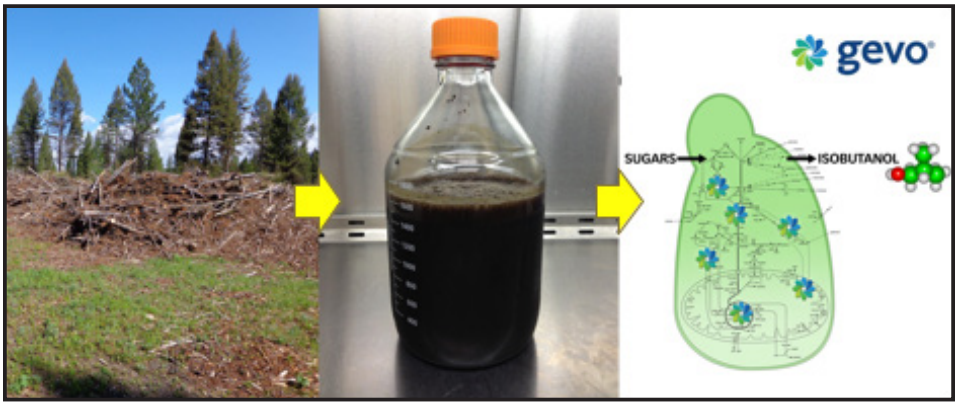
Two pretreatment methods were used in this study: dilute acid (DA) and alkaline peroxide (AP). After pretreatment, a smaller degree of carbohydrate variation existed between pretreated samples (9%) compared to the carbohydrate variation observed in the raw non-pretreated biomass (16%). The authors point out that pretreatment has a "normalizing" effect on the carbohydrate levels; because pretreated material contains all of the carbohydrates available for further downstream release, the carbohydrate variation detected in the raw biomass is an unlikely indicator of how much carbohydrate will be available to convert to biofuels and other products.

When the pretreated samples were subjected to hydrolyzing enzymes, the resulting carbohydrate (sugar) yields showed the high degree of variation. Sugar yields from DA pretreated biomass after enzymatic hydrolysis ranged from 12.6 to 46.1%; yields from the AP pretreated material after hydrolysis ranged from 6.31 to 24.2. These yields will vary depending on the severity of the pretreatment, but the wide degree of variation is the significant factor.

Because pretreatment and enzymatic hydrolysis can cause variation in carbohydrate yield, the authors developed a "recalcitrance factor" that incorporates both pretreatment and hydrolysis results into a yield value for the overall conversion process.

Future Direction

Breeding softwood trees with improved sugar yields is a long-term prospect, well beyond NARA's 5-year project duration. The results from this study indicate that recalcitrance variation does exist among Douglas-fir individuals. This data will be paired with genomic data taken from the same samples and used to identify identifying genetic markers that influence the level of recalcitrance. Those genetic markers should allow breeders to develop a future generation of plantation trees that produce residual material more suitable to chemical conversion.



Conversion process from forest residuals to hydrolysate to isobutanol. Images courtesy of Gevo, Inc

Converting Wood Simple Sugars to Isobutanol

Gevo, a NARA affiliated organization, has developed a fermentation and process technology that converts biomass sugars into isobutanol and further into biojet fuel through chemical processing. To convert the simple sugars found in wood to isobutanol, they rely on a technology called GIFT®, Gevo Integrated Fermentation Technology. In GIFT®, specialized yeast are used as a biocatalyst designed to ingest the simple sugars, produce isobutanol and then excrete the isobutanol into a broth. Natural yeast primarily produce ethanol during fermentation. The production of isobutanol over ethanol provides commercial advantages for fuel refiners.

Read Gevo's latest white paper, *Isobutanol for Transportation Fuels*

Characterizing Hydrolysate

Gevo's work to producing isobutanol from wood sugars has been a multi-step process. The first task was to characterize the wood derived sugar solutions (commonly called hydrolysate) fed to the yeast biocatalysts. Liberating simple sugars from softwoods requires a pretreatment and enzymatic hydrolysis step. Pretreatment is intended to break apart the wood fibers so that the sugar polymers are exposed. The sugar polymers are then decomposed to sugar monomers by addition of commercial enzymes. Wood is naturally resistant to breakdown. Consequently, the pretreatment process involves harsh conditions that can produce a variety of compounds. Some of these compounds are toxic to yeast and can inhibit yeast growth and productivity.

Gevo scientists measured the amount and type of simple sugars and inhibitors present in hydrolysate from multiple Douglas-fir and forest residue samples subjected to different pretreatment protocols. Turns out that sugar and inhibitor yields vary significantly depending on the quality of the wood feedstock and pretreatment protocols.

Measuring Yeast Biocatalyst Growth and Isobutanol Production

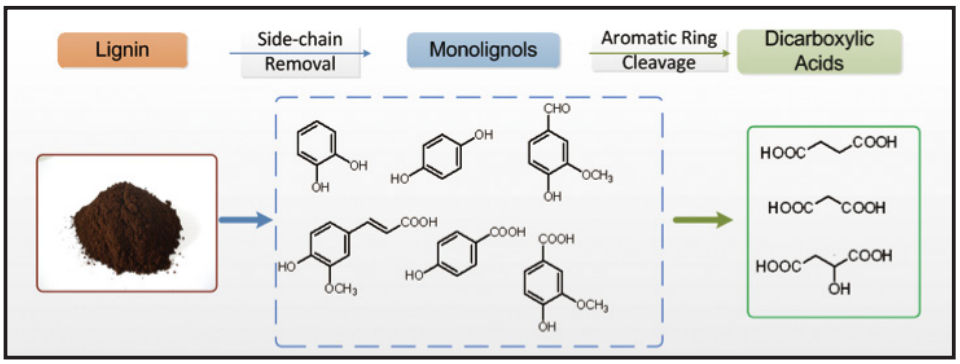
After the various hydrolysates were characterized, Gevo measured the growth and isobutanol production rates for their yeast biocatalyst at increasing concentrations of hydrolysate. Their results indicated that the pretreatment protocol has an effect on growth and isobutanol production rates and established the initial hydrolysate concentrations for optimal productivity. These results also contributed to NARA's downselect decision to use the mild bisulfite pretreatment protocol

over other pretreatments as the preferred pretreatment process to convert forest residuals into biojet fuel.

Adapting the Yeast Biocatalyst to Douglas-fir Hydrolysate

Taking advantage of yeast's rapid growth rate and of the process of natural selection, Gevo is currently adapting their yeast biocatalyst to the Douglas-fir residual hydrolysate pretreated by the mild bisulfite protocol. The adaptation process involves isolating the best performing yeast strains over multiple generations. In the next two years, Gevo will contribute to NARA and scale-up and optimize the GIFT® fermentation process on Douglas-fir hydrolysate using the adapted yeast biocatalyst strains.

View a research poster on Gevo's contribution to the NARA project



The progression of lignin conversion to dicarboxylic acid. Image courtesy of Dr. Xiao Zhang, WSU.

Producing Dicarboxylic Acids from Biorefinery Lignin

NARA supports research that discovers ways to develop commercially valuable products from forest residuals. When forest residuals are converted to biojet fuel, a significant amount of the material remains. That remaining material is a mixture of many byproducts from the conversion process, but the predominant component is lignin.

NARA researchers are investigating using this lignin-rich material to make activated carbon products for mercury absorption and super capacitors, asphalt dispersants, thermoplastics and epoxy resins. Generating commercial products from the lignin-rich material will add value to the forest residuals and improve the economics of a wood residual to biojet fuel supply chain.

In a recently published paper funded by NARA, Washington State University-Tri Cities researcher Xiao Zhang and his team describe a novel method to selectively produce dicarboxylic acids from softwood lignin.

Read *Selective Conversion of Biorefinery Lignin into Dicarboxylic Acids*

Multiple Dicarboxylic Acids (DCA) are Produced

The technology developed by Dr. Zhang and his team uses the mineral chalcopyrite and hydrogen peroxide to producing DCAs including malonic acid, succinic acid, malic acid and maleic acid. These acids are used commercially to produce biopolymers, pharmaceuticals and food additives. Not only did Dr. Zhang's team produce the dicarboxylic acids, but they also investigated how the reaction proceeds from lignin to DCA. The initial lignin-to-DCA conversion yields were low (14% based on carbon) in this study, but yields should increase with further optimization of the reaction conditions. The team is currently working with an industrial partner to design and test a two-step reaction system to improve the DCA yields.

In addition to the experimental work, Dr. Zhang's team is developing a techno-economic model to evaluate the feasibility of this technology.

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