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Novel System for Recalcitrance Screening Will Reduce Biofuels Production Costs

The most significant barrier to the economical conversion of cellulosic biofuels is biomass recalcitrance—the difficulty of converting complex structural plant material to fermentable sugars. Researchers at the National Renewable Energy Laboratory (NREL), working with scientists from the University of California-Riverside (UCR), developed a high-throughput (HTP) platform capable of identifying biomass samples with uncommon cell wall chemistry and conversion characteristics, including those that would be easiest to convert to fermentable sugars. The custom-designed reactor system is the first truly HTP pretreatment and enzyme digestion system for biomass conversion research.

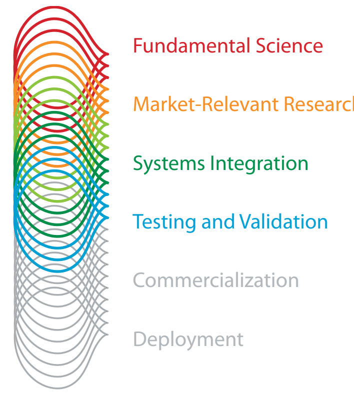
This new system will allow researchers to much more rapidly screen large numbers of samples and identify the most promising biomass feedstocks for higher efficiency and lower cost biofuels conversion processes. NREL will be screening thousands of variants of different biomass feedstocks to link genetic traits with environmental factors that can enhance biomass conversion efficiencies. Identifying the genes controlling the anatomical, chemical, and morphological features of biomass is essential to develop the next generation of low-cost, easily convertible biomass feedstocks.

To identify superior performing biomass feedstocks using approaches that account for natural diversity and randomness, researchers must measure the cell wall chemistry and recalcitrance in a very large number of samples under identical conditions—a job that is prohibitively expensive and time-consuming when using traditional analysis techniques. No equipment existed for rapid, HTP screening of biomass recalcitrance, so NREL scientists set to work developing their own system.

The Challenge of HTP Biomass Analysis

Through years of research, NREL has developed an extensive suite of precise laboratory analytical procedures for measuring biomass feedstock characteristics. However, these traditional wet chemistry techniques are labor-intensive and time consuming—the complete set of tests takes nearly two weeks to complete, involves numerous analysts, and can require large quantities of samples and chemical reagents. High-throughput robotics platforms have been used in the pharmaceutical and other biotechnology industries since the 1990s. These automated platforms use small sample sizes and can analyze hundreds or thousands of samples at a time. While biomass conversion research is more difficult to automate because of nonuniform feedstock characteristics and extreme process conditions, such as high temperatures and low pH, these complications have been overcome by adapting existing HTP technologies.

The Spectrum of Clean Energy Innovation



Through deep technical expertise and an unmatched breadth of capabilities, NREL leads an integrated approach across the spectrum of renewable energy innovation. From scientific discovery to accelerating market deployment, NREL works in partnership with private industry to drive the transformation of our nation's energy systems.

This case study illustrates NREL's contributions in Fundamental Science through Testing and Validation

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NREL is a national laboratory of the U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy, operated by the Alliance for Sustainable Energy, LLC.

The NREL and UCR scientists found that the key to a successful HTP system was to perform the pretreatment, enzyme hydrolysis, and sugar assay steps all within the same vessel. To accomplish this, the researchers adapted solids-dispensing robots to distribute biomass into custom 96-well reactor plates, and they developed a stackable plate steam reactor capable of simultaneously pretreating 1,920 biomass samples. NREL's HTP reactor plates were custom-fabricated from aluminum to withstand high pretreatment temperatures and gold-plated for corrosion resistance. NREL researchers also customized the plates by boring steam channels between each of the wells so steam and cooling water can circulate evenly through the reactor, resulting in uniform reaction conditions for all samples. Another key development was effectively sealing the reactors during pretreatment while maintaining robotic access to the samples inside the plate.

After pretreatment, enzymes are added into the sample wells and the plates are incubated for three days while the enzymes break down the biomass into its component sugars. The pretreatment and digestion conditions are defined to yield 50% to 70% cellulose conversion, well above the conversion levels of raw material, yet below high conversion levels that could mask sample variability and bias the analysis. The resulting liquid hydrolysate is assayed for glucose and xylose, the primary sugars that result from enyzme hydrolysis.

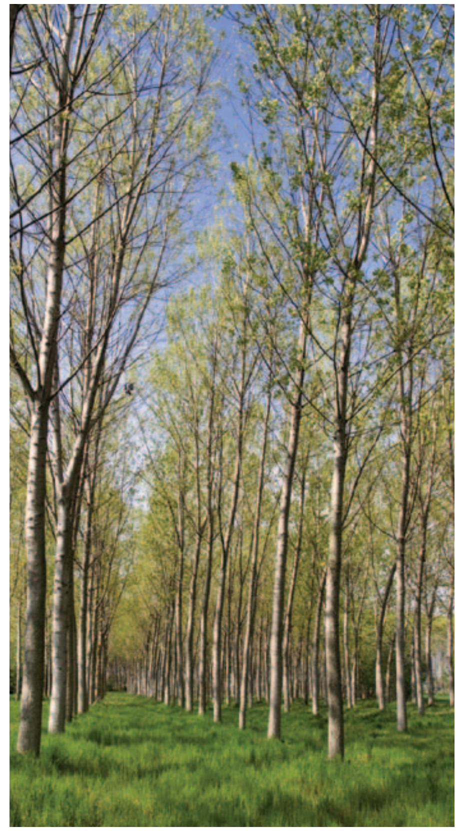
Putting the System to Use: Screening Poplar Tree Strains for Conversion Efficiency

To demonstrate the new HTP platform, NREL researchers screened 755 poplar core samples from the Pacific Northwest as part of an ongoing U.S. Department of Energy BioEnergy Science Center study. Partial compositional analysis was performed on each sample by HTP pyrolysis molecular beam mass spectroscopy, which provided information about the chemistry of the cell wall, including the ratios of cellulose, hemicellulose, and lignin.

The sugar release data from the poplar experiment provides a general idea of how the recalcitrance varies within a large set of samples. In this case, there was a large variation in conversion efficiency, more than 25%, which correlated with the chemical composition of the samples. Therefore, trends in recalcitrance were identified with respect to a number of measurable characteristics, including the ratio of certain lignin species known as syringyl and guaiacyl. The researchers found that the sugar release was independent of overall lignin content, but that both the glucose and the xylose release increased with increasing syringyl:guaiacyl ratio.

The results of NREL's HTP chemical and conversion analyses will be combined with genetic information gathered by other BioEnergy Science Center researchers to discover the factors contributing to lower biomass recalcitrance. There is a large variation in physical and chemical characteristics that can be exploited to develop improved biomass feedstocks. This information provides the scientific basis for the rational development of improved poplar trees and other potential feedstocks that can lead to significant reductions in the cost of biofuel production.

NREL's custom-designed reactor system, a high-throughput pretreatment and enzyme digestion system, allows researchers to more rapidly screen large numbers of samples to identify the most promising biomass feedstocks. PIX 17132



To demonstrate the new high-throughput platform, NREL screened and performed partial compositional analysis on 755 poplar core samples from the Pacific Northwest. iStock

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