

Research Advances

**NREL
Leads the
Way**

Cellulosic Ethanol

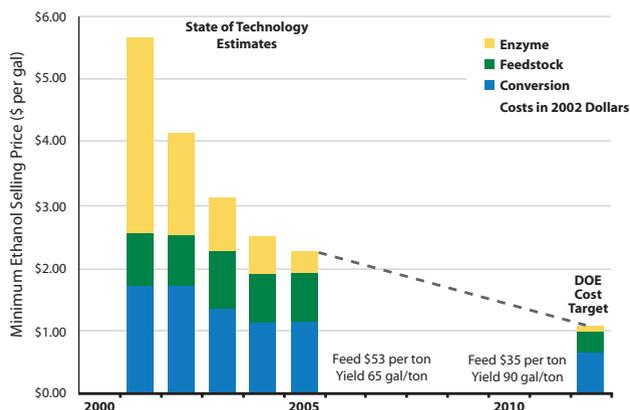
Fueling the Future

On the road to energy security

One of our greatest challenges is to reduce our nation's dependence on imported petroleum. To accomplish this, we need a variety of alternative fuels, including ethanol produced from cellulosic materials like grasses and wood chips. Fortunately, the United States has abundant agricultural and forest resources that can be converted into biofuels. Recent studies by the U.S. Department of Energy (DOE) suggest these resources can be used to produce enough ethanol – 60 billion gallons/year – to displace about 30% of our current gasoline consumption by 2030.

How do we get there? Currently, there are no commercial cellulosic ethanol refineries. The ethanol we use is derived primarily from corn kernels, a form of starchy biomass. When manufacturers produce ethanol from corn, they use enzymes to convert starches to simple sugars and yeasts to ferment the sugars into ethanol. Cellulosic biomass contains sugars as well, but they are much harder to release than those in starchy biomass. To complicate matters, the process of releasing the sugars produces by-products that inhibit fermentation, and some of the sugars from cellulosic biomass are difficult to ferment.

All this makes cellulosic ethanol production complicated—and expensive. To displace petroleum, cellulosic ethanol must be cost competitive. DOE has determined that competitiveness can be achieved at an ethanol production cost of \$1.07/gallon (in 2002 dollars)

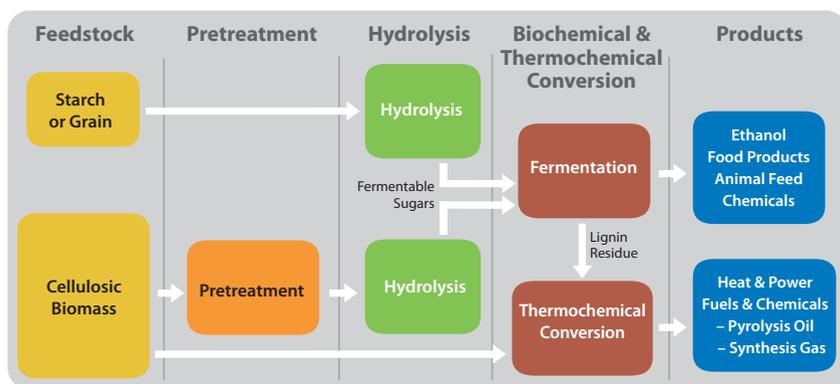


NREL has led progress toward DOE's cost target of \$1.07/gallon for biochemically produced ethanol. Similar progress is being made for thermochemically produced ethanol.

and aims to achieve this goal by 2012. To do this, the technology used to produce cellulosic ethanol must be improved. That's where the National Renewable Energy Laboratory (NREL) comes in.

NREL leads DOE's National Bioenergy Center and is on the cutting edge of cellulosic ethanol technology. NREL's research addresses each step of the processes that produce cellulosic ethanol and valuable co-products. NREL's research covers the full spectrum from fundamental science and discovery to demonstration in fully integrated pilot plants.

This brochure highlights some of NREL's recent advances in cellulosic ethanol production. Research at NREL addresses both biochemical (chemicals, enzymes, and fermentative microorganisms) and thermochemical (heat and chemical) processes. For the biochemical processes, NREL investigates pretreatment, hydrolysis, and fermentation steps as well as process integration and biomass analysis. For the thermochemical processes, NREL researches catalyst development, process development, and process analysis.



Schematic of a hypothetical integrated ethanol biorefinery.

Cover image: Confocal laser microscope image of rind tissue in corn stover, showing the detailed structure of two vascular bundles.



Pretreatment

Improving the critical first step toward cost-competitive ethanol

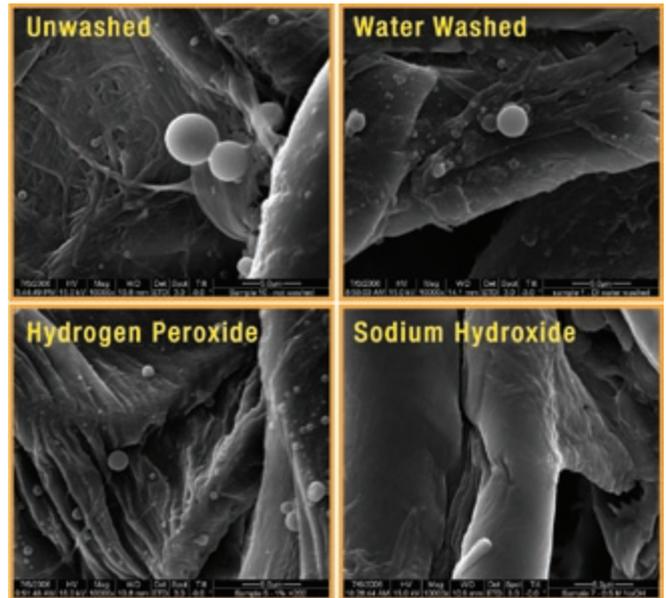
To break down cellulose—the primary source of sugar in fibrous biomass—you have to first get past hemicellulose and lignin, which surround the cellulose in

a protective sheath. This is the job of pretreatment. NREL typically uses a moderately high-temperature, high-pressure dilute acid pretreatment process to break down (hydrolyze) hemicellulose and disrupt or dissolve lignin. Hydrolyzing the hemicellulose also creates another important source of soluble sugars for later fermentation to ethanol.

NREL is investigating potentially cheaper, but still effective, pretreatment methods. In one recent advance, NREL researchers applied their knowledge of biomass structural changes to pretreatment process development. Lignin dissolved under certain pretreatment conditions can apparently redeposit onto cellulose, creating a barrier to effective cellulose hydrolysis and reducing sugar yield. NREL is using its state-of-the-art imaging and analytical tools to understand lignin redeposition and design pretreatment processes that minimize its detrimental effects.

In another recent advance, NREL employed enzymes to enable milder pretreatment. Although dilute acid pretreatment can break down hemicellulose very effectively, the severe conditions require expensive processing equipment and tend to degrade the sugars. Using a milder pretreatment process could cut process costs dramatically and eliminate sugar degradation losses. The challenge is to maintain a high level of effectiveness with the milder process, which is accomplished by using enzymes to further break down the hemicellulose after pretreatment. NREL has shown that proper mixtures of enzymes can enhance hemicellulose hydrolysis. In an experiment on pretreated corn stover, adding a hemicellulase enzyme to break down the hemicellulose increased the yield of xylose (a sugar resulting from hemicellulose hydrolysis) by 12% across a range of pretreatment conditions. Breaking down the hemicellulose also enhanced cellulose hydrolysis, resulting in a 6% higher glucose yield.

To get a broader look at pretreatment options, NREL participates in the Biomass Refining Consortium for Applied Fundamentals and Innovation (CAFI). Each

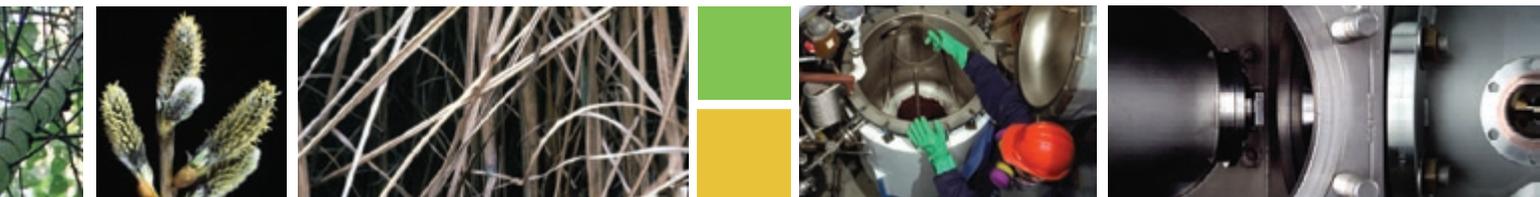


NREL used scanning electron microscopy to reveal what are thought to be lignin droplets remaining on pretreated filter paper after washing with various solvents. (T. Vinzant, NREL)

CAFI participant is evaluating a different pretreatment approach using standardized experimental design and data reporting protocols. The CAFI projects allow participants to compare pretreatment and downstream process performance across a range of lignocellulosic feedstocks. NREL is using this knowledge to help identify the best pretreatment approaches for near- and long-term biorefining platforms. (For more information on CAFI, see “Coordinated Development of Leading Biomass Pretreatment Technologies,” *Bioresource Technology*, December 2005.)



This pretreatment reactor hydrolyzes hemicellulose and solubilizes some lignin. The pressurized hot wash process separates these materials before they can reprecipitate. This system uses dilute sulfuric acid at increased temperature and pressure, but pressurized hot wash may work well with any pretreatment system.



Enzymatic Hydrolysis

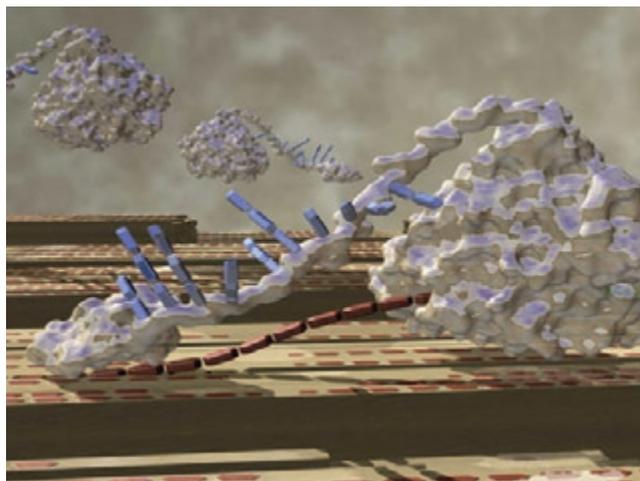
Unlocking the full potential of cellulosic biomass

Plants have evolved over several hundred million years to be recalcitrant—resistant to attacks from the likes of bacteria, fungi, insects, and extreme weather.

Breaking down plants is no easy task. For cellulosic ethanol production, the primary challenge is breaking down (hydrolyzing) cellulose into its component sugars.

NREL is exploring the causes of biomass recalcitrance and ways to overcome it using cellulases (enzymes that break down cellulose). The goals are to maximize the conversion of cellulose to sugar, accelerate the rate of conversion, and use fewer, cheaper enzymes. NREL's recent advances include employing state-of-the-art capabilities to characterize plant structure and developing superior enzymatic hydrolysis processes.

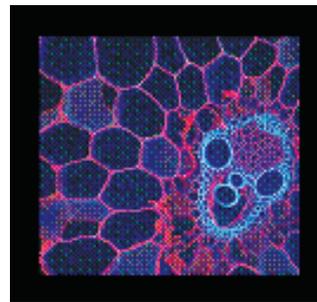
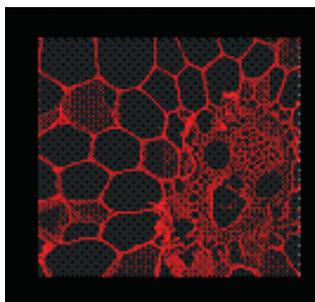
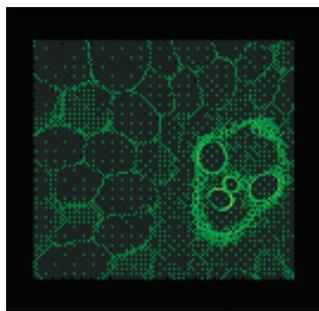
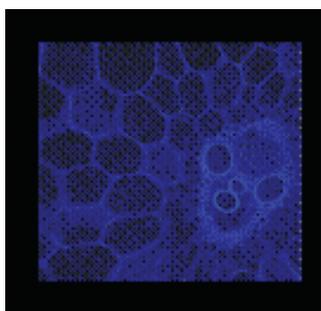
To make contact with cellulose, the enzymes must get past a complex maze of plant structures. NREL is mapping this labyrinth as a first step toward overcoming it. A unique array of microscopy tools and techniques in NREL's new Biomass Surface Characterization Laboratory enables researchers to image plant structures down to the molecular level. To probe even further—visualizing structures and processes at scales that cannot (yet) be observed—NREL and its partners are building a sophisticated molecular dynamics model of the cellulose-cellulase system. When complete, it will be the largest biological computer model ever developed.



Advanced computer modeling capabilities help NREL understand and improve enzymatic hydrolysis processes. This illustration of the CBH I enzyme is based on models developed by NREL.

Once cellulases make contact with cellulose, the real work begins. Cellulases act very slowly. That's why dead trees take years to decompose in the forest. To accelerate cellulose conversion, it is critical to start with the best enzymes nature has to offer. The most active known cellulases are in the cellobiohydrolase I (CBH I) family, derived from fungi. But not all CBH I enzymes are equal. NREL recently confirmed the existence of CBH I enzymes that are twice as active as those from industrial sources.

NREL and its partners Genencor International and Novozymes have developed a “cocktail” of cellulases to improve hydrolysis. In combination with NREL's process development improvements, this advance has reduced enzyme cost twentyfold. This work received an R&D 100 Award in 2004.



These ultra-sharp laser microscope images were created with the Biomass Surface Characterization Laboratory's scanning confocal microscope, which can be used to build 3-D representations of plant structures. (S.Porter, NREL)



Fermentation

Creating “super-bugs” for superior ethanol yield

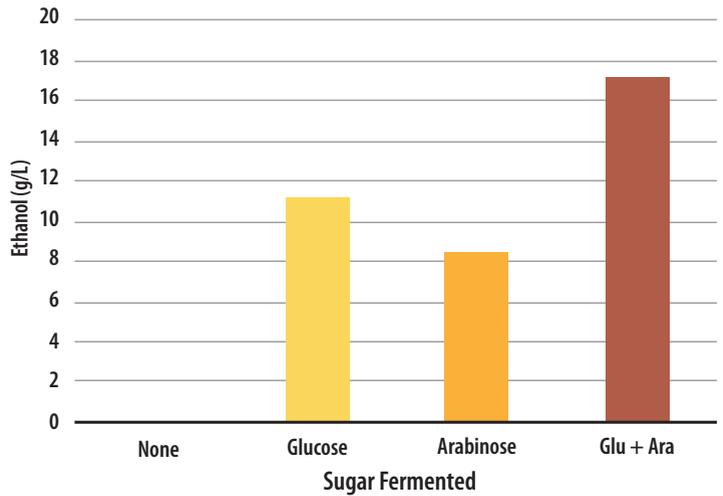
During fermentation, microorganisms (primarily fungi and bacteria) convert the sugars in biomass to ethanol. Under ideal conditions, these “bugs” will work contentedly, consuming sugars and producing

ethanol and other products. But conditions in a cellulosic ethanol biorefinery are anything but ideal.

The hot soup—called a hydrolyzate—generated after pretreatment and hydrolysis contains not only fermentable sugars, but also compounds (such as acetic acid) that are toxic to the bugs. Other things that are toxic in the fermentation process and the hydrolyzate are a high-solids concentration and a rising ethanol concentration. Because microorganisms found in nature do not function well in this hostile environment, NREL is creating “super-bugs” that thrive in it.

Yeasts are currently the fermentation organisms of choice for the corn ethanol industry. They are reasonably tolerant of ethanol, acid, and moderately high temperatures. However, existing yeast strains cannot withstand highly toxic hydrolyzates or ferment 5-carbon sugars and minor 6-carbon sugars efficiently. NREL, along with the National Corn Growers Association (NCGA) and Corn Refiners Association (CRA), developed yeast capable of fermenting a particular 5-carbon sugar, arabinose, which constitutes up to 20% of the fermentable sugars in corn fiber. Three genes from a bacterium were inserted into the yeast *Saccharomyces cerevisiae*. This work resulted in the first ever demonstration, in 2000, of arabinose fermentation by yeast. Next, NREL plans to test the strain under real biorefining conditions—in the hydrolyzate.

NREL also pioneered the use of a yeast alternative, the bacterium *Zymomonas mobilis* (Zymo). Zymo gives a high ethanol yield and tolerates high ethanol concentrations. Using genetic and metabolic engineering, NREL developed acetic acid-tolerant Zymo strains that can ferment arabinose and the most important 5-carbon sugar, xylose. This strain resulted in several patents and an R&D 100 Award. NREL also pioneered a technique to make the Zymo strain stable (the bacteria’s offspring have the same genes as the parents) by inserting key genes into the genome. NREL’s Zymo work has included successful collaborations with the NCGA and CRA, the chemical company Arkenol (now BlueFire Ethanol), and DuPont.



The yeast strain developed by NREL, NCGA, and CRA is the world’s first yeast to ferment arabinose. These results show an ethanol yield of 83% from arabinose in a defined medium (not a hydrolyzate). From left to right, initial sugar concentrations were 0 g/L, 20 g/L glucose, 20 g/L arabinose, and 20 g/L glucose + 20 g/L arabinose. Expected ethanol from 20 g/L of sugar is 10.2 g/L at 100% yield. (A. Singh, NREL, patent pending)



Fermentation processes are tested in NREL’s biochemical process development unit (PDU). This 9,000-L fermenter is large enough to produce sufficient lignin for processing in the downstream thermochemical PDU.



Process Integration

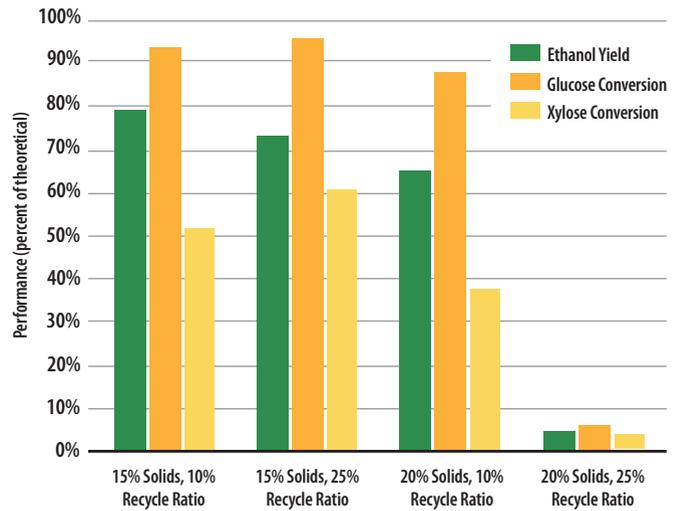
Tying together the integrated biorefinery

To produce low-cost ethanol, biorefineries will need to link the refining steps into an integrated process. However, optimizing conditions in one step of the process can influence performance in other steps. The challenge is to find the right combination of trade-offs that optimize the integrated process. Studying integrated biorefinery operations requires an advanced process development unit and state-of-the-art chemical analysis capabilities. NREL's research on high-solids operation is a good example.

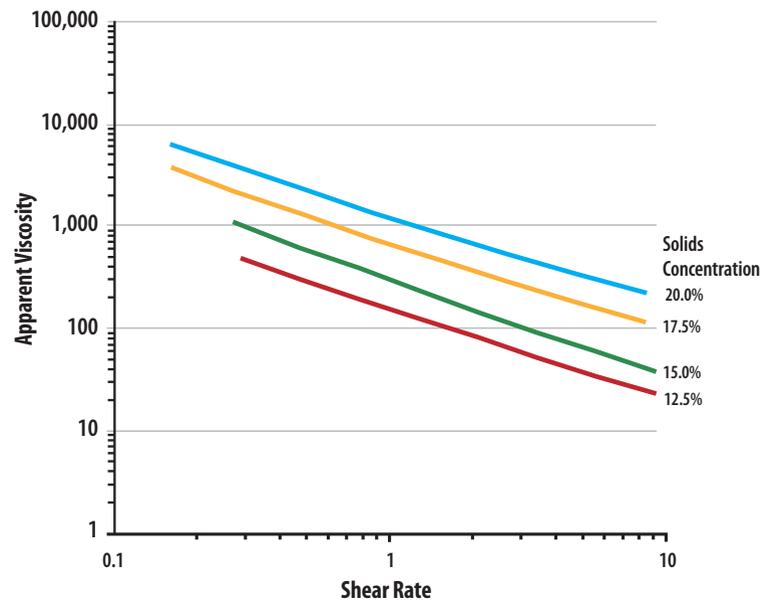
NREL has shown that high-solids operation—using a high ratio of biomass to water in the biorefining process—is one key to cutting ethanol costs. The less water introduced in the pretreatment step, the higher the potential sugar concentration and the less equipment and energy the process requires. The result is lower-cost ethanol. In a perfect world that would be enough, but high solids concentrations can create problems elsewhere in the process.

In a first-of-its-kind study, NREL demonstrated that a moderately high solids concentration combined with recycled process water severely inhibits fermentation and, consequently, lowers ethanol yield. This is important because commercial biorefineries will need to recycle water, taking it from the back end of the refining process and combining it with fresh water at the front end of the process. NREL's study identified the ability to achieve high solids concentration and high levels of process water recycle as an issue that must be considered in both pretreatment and fermentative microorganism development.

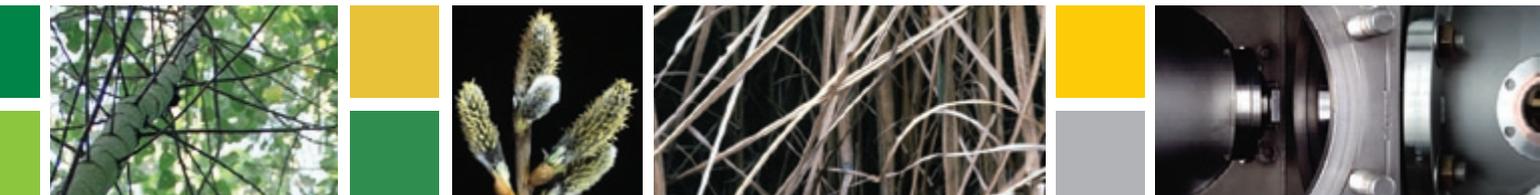
Managing the properties of high-solids mixtures is another process integration challenge. Like adding flour to water in a recipe, adding biomass to water makes the mixture thicker and more viscous. This has important implications for the efficient flow and conversion of biomass through the integrated process. NREL is developing unique capabilities in biomass rheology—the science of the deformation and flow of materials—to determine the best ways to manage high-solids biomass mixtures. This research is critical to providing process engineers with rheological information needed to design a commercial biorefinery.



Pioneering NREL research shows ethanol yield dropping dramatically at moderately high solids concentration and high recycle ratio (the ratio of recycled water to fresh water). (D. Schell, NREL)



NREL is quantifying the rheological properties of various biomass materials. These results show how the viscosity (resistance to flow) of pretreated corn stover increases with higher solids concentration and decreases with faster shear rate (speed of mixing). (J. McMillan, NREL)



Thermochemical Conversion

Honing a powerful path to economic ethanol

The advances described up to this point relate to biochemical conversion of carbohydrates to ethanol. However, ethanol can also be produced thermochemically

from any form of biomass. In this approach, heat and chemicals are used to break biomass into syngas (CO and H₂) and reassemble it into products such as ethanol. This method is particularly important because up to one third of cellulosic biomass—the lignin-rich parts—cannot be easily converted biochemically.

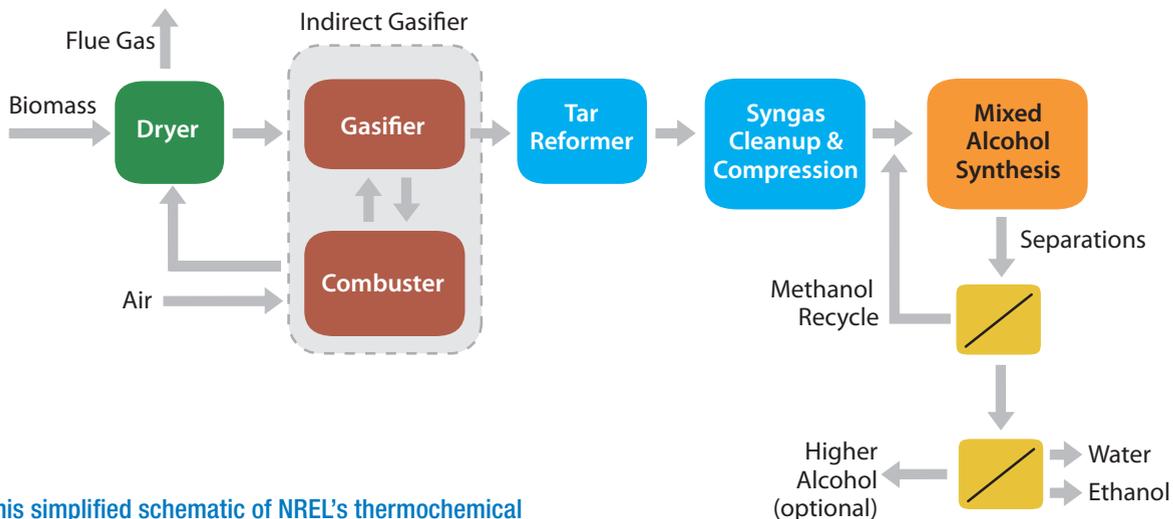
Forest products and mill residues typically have high lignin contents, making them unattractive feedstocks for biochemical conversion yet suitable for thermochemical conversion. In an integrated biorefinery, lignin-rich residues from the biochemical process could also be converted thermochemically.

A thermochemical biomass conversion process is complex, and uses components, configurations, and operating conditions that are more typical of petroleum refining. And, just like researchers in the petroleum industry, NREL uses a combination of experimental research together with process economic models to explore a large number of possible process configurations. A much simplified

schematic of NREL's preferred configuration is shown below. This configuration employs an indirect gasifier, tar reforming, and a mixed alcohol synthesis step designed to maximize ethanol yield.

In thermochemical conversion, biomass is converted into syngas, and syngas is converted into an ethanol-rich mixture. However, syngas created from biomass is not “clean”—it contains contaminants such as tar and sulfur that interfere with the conversion of the syngas into products. These contaminants must be removed. NREL has developed tar-reforming catalysts and catalytic reforming processes that have demonstrated high levels of tar conversion—converting up to 97% of the tar into more syngas. This not only cleans the syngas, it also creates more of it, improving process economics and ultimately cutting the cost of the resulting ethanol. NREL has also made progress regenerating the tar-reforming catalyst after it has been partially deactivated by sulfur poisoning.

NREL is evaluating many different process options and their associated costs to help identify key barriers to low-cost ethanol production. For example, process models indicate that reducing tars and hydrocarbons from syngas can decrease the production cost of ethanol by 33%. NREL models also highlight the need for extensive heat integration and quantify performance targets needed to achieve DOE's ethanol cost goals thermochemically.



This simplified schematic of NREL's thermochemical conversion model shows the many steps that can be manipulated to optimize efficiency and cost.



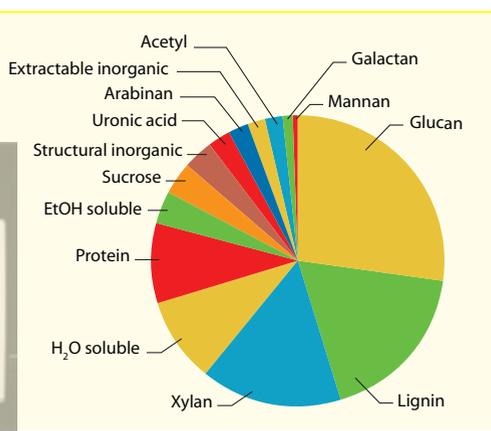
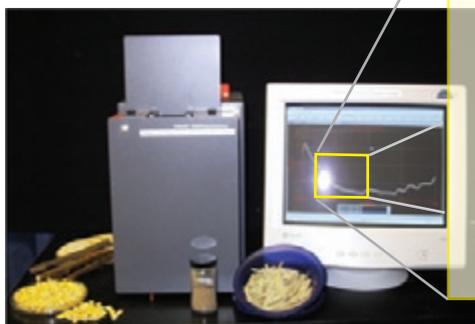
Biomass Analysis

Enabling total control of ethanol production

You can't control what you can't measure. Biorefinery operators will need to know the precise composition of the biomass going through their processes so they can tightly control the cost and quality

of the products coming out. The faster and more reliable the measurements are, the better and cheaper the final products will be.

NREL's capabilities are constantly evolving to meet industry's need for accurate and rapid biomass analysis. Researchers recently developed a way to rapidly analyze biomass composition using near-infrared (NIR) spectroscopy. In this technique, light reflected off a biomass sample is analyzed to determine the sample's composition. Compared with traditional

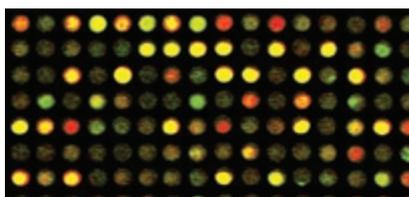


wet chemistry analysis, this is a huge leap forward. Analyzing a sample with this new approach takes minutes instead of weeks and costs tens of dollars instead of thousands of dollars—without sacrificing precision or accuracy. The rugged NIR instruments can even be adapted to a working biorefinery to measure, for example, the chemical composition of corn stover as it enters and exits pretreatment.

What if you could measure an adult plant's composition while it is still a sprout? You could grow plants that yielded the most ethanol, maximize cellulose content, and minimize lignin. Or what if, by knowing how genes affect plant composition, you could create the ideal ethanol feedstock? NREL's analytical capabilities are making these scenarios a reality. Using techniques such as molecular beam mass spectrometry and nuclear magnetic resonance spectroscopy, NREL is measuring cell wall chemistry

more quickly and accurately than anyone else in the world. These measurements are used to predict adult plant composition. In 2006, NREL analyzed the cell wall chemistry of more than 10,000 samples for industry and university partners. These thermoanalytical capabilities can help accelerate crop breeding and genetic engineering.

NREL's NIR spectroscopy technique rapidly analyzes biomass composition and can be adapted to on-line use.



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Please see the following Web sites for more information on biomass and related research at NREL:

NREL's R&D "Working With Us" Web page: www.nrel.gov/biomass/workingwithus.html

NREL's Biomass Research Web site: www.nrel.gov/biomass/

NREL's Chemical & Biosciences Center Web site: www.nrel.gov/basic_sciences/

DOE Biomass Program Web site: www.eere.energy.gov/biomass/

The National Bioenergy Center is headquartered at NREL:

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