Tall order. Starches and sugars in corn kernels readily ferment into alcohol; corn stalks are more challenging.

CELLULOSIC ETHANOL

Biofuel Researchers Prepare To Reap a New Harvest

After decades in the background, technology for converting agricultural wastes into liquid fuels is now poised to enter the market

When U.S. President George W. Bush announced an initiative in January to reduce U. S. gasoline use by 20% in 10 years, critics could be forgiven for thinking it sounded familiar. Presidents since Jimmy Carter have called for reducing U.S. dependence on foreign oil. But so far there's been little to show for it. Shale oil, electric cars, and hydrogen fuel cells have all at one time or another had their 15 minutes of fame. But all have failed to make a dent in U.S. gasoline use.

Today, biofuels are the alternatives du jour, with ethanol chief among them. And in the United States, that currently means corn ethanol. But the big hope for the field is a technology called "cellulosic ethanol," which aims to turn all kinds of plant material—from corn stalks and wheat straw to forest trimmings—into fuel. According to a 2005 study by the U.S. departments of Energy and Agriculture, the U.S. could convert 1.3 billion dry tons a year of biomass to 227 billion liters (60 billion gallons) a year of ethanol with little impact on food or timber harvests and in the process displace 30% of the nation's transportation fuel. Not bad for what amounts to a lot of unwanted yard waste.

No commercial cellulosic-ethanol plants exist today. But despite the failures of previous alternative fuels, decades of research in biotechnology, chemistry, and chemical engineering are merging to bring cellulosic-ethanol technology to the verge of a payoff. A host of small and large chemical companies have jumped into the area, propelled by recent high gas prices and nearly $2 billion in private and venture-capital funding for biofuels last year alone, according to London-based research firm New Energy Finance. A handful of cellulosic-ethanol demonstration plants have popped up as a result. And last month, the U.S. Department of Energy (DOE) announced awards of $385 million for six commercial-scale cellulosic-ethanol refineries (see table, p. 1489) that are expected to produce more than 130 million gallons of ethanol per year.

That's still just a small fraction of the some 5 billion gallons of corn-based ethanol produced in the U.S. annually. But confidence in the new technology is riding high. Experts believe that scientific successes are now coming in a steady stream, which should progressively improve the technology and chip away at ethanol prices.

"I think we will be there with cellulosic ethanol much more quickly than anybody realizes," says Bruce Dale, a chemical engineer at Michigan State University (MSU) in East Lansing who has worked on ethanol conversion technology for 30 years.

Fuel versus food?

Ethanol hasn't always been an alternative fuel. Henry Ford originally planned to use it to power his Model T's. But it was quickly supplanted by cheap and plentiful gasoline, which packs 30% more energy per gallon than ethanol does.

Ethanol began making its comeback after the oil shocks of the 1970s. Brazil launched a national effort to convert sugar cane into ethanol in 1975 in hopes of reducing its vulnerability to high oil prices. As part of that effort, the country's federal government required gas stations to blend 25% ethanol into gasoline and encouraged carmakers to sell engines capable of running on pure ethanol. As a result, ethanol production in Brazil has climbed steadily, from 0.9 billion gallons in 1980 to 4.2 billion gallons last year. And the price of the fuel has dropped steadily to $0.81 cents a gallon, according to a recent article by José Goldemberg, the State of São Paulo's Secretary for the Environment (*Science*, 9 February, p. 808).

U.S. ethanol producers have seen a similar surge in output. In 2005, they turned out roughly 4 billion gallons of ethanol, or about 3% of the 140 billion gallons of gasoline used in the U.S. each year. Today, most of that ethanol is blended with gasoline at a 10:90 ethanol-to-gasoline ratio to boost the fuel's octane rating, which allows it to burn more cleanly, reducing urban smog. Two years ago, Congress mandated a production increase to 7.5 billion gallons a year by 2012. And the president's recent initiative aims to produce as much as 35 billion gallons of alternative fuels by 2017. The European Commission too has called for 10% of its transportation fuel to come from biofuels such as ethanol and biodiesel by 2020.

But crops such as corn and sugar cane won't be enough to produce all this fuel. According to one recent DOE study, U.S. corn grain ethanol production is likely to top out somewhere around 12 billion gallons a year. "Even if you took all the starch and converted it to fuel, it only gets you to about 10% of our gasoline," says Jim McMillan, a biochemical engineer with the

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National Renewable Energy Laboratory (NREL) in Golden, Colorado. Long before that point, diverting too much of the corn crop would cause dramatic rises in the cost of the food. And even at today's modest levels of ethanol production, a price pressure is already being felt. Corn prices in the United States hit a 10-year high of $4.47 a bushel ($176 per metric ton) last month, nearly double the price a year ago, fueled in part by the increased demand for ethanol.

To get past the food-versus-fuel debate, "you've got to get into cellulose," says McMillan. Doing so would both increase the volume of ethanol that can be made and lower emissions of greenhouse gases. That's where cellulosic ethanol really shines, says Alexander Farrell, an energy resource expert at the University of California, Berkeley. In a paper published last year in *Science* (27 January 2006, p. 506) and in follow-on work, Farrell and colleagues found that because of its high energy inputs, using corn-based ethanol instead of gasoline reduces greenhouse gas emissions only about 18%. With its modest energy inputs, cellulosic ethanol fares much better, reducing greenhouse gas emissions by 88%.

Sweet science

But converting cellulose to fuel is far more difficult than starting with simple sugar, as in Brazil, or corn starch, as in the United States. Starch is a straightforward polymer of glucose that is easily broken down by enzymes. Agricultural and forest wastes, by contrast, are far more complex. This biomass is made up of three ingredients: cellulose, a polymer of the six-carbon sugar glucose that's the main component of plant cell walls; hemicellulose, a branched polymer composed of xylose and other five-carbon sugars; and lignin, which crosslinks the other polymers into a robust structure.

To convert any source of sugars to ethanol, those sugars must first be made accessible. That's simple in the case of sugar cane, where the sugar is harvested and made into a syrup. It's a bit harder with corn grain. But there, engineers simply add enzymes called amylases to clip apart the starch polymer into separate glucose molecules. But with other agricultural products, such as leaves, stalks, grasses, and trees, the material must be broken down so that crystalline fibers made up of hemicellulose and cellulose can be digested into simple sugars before being turned over to microbes that convert them to ethanol, a process known as fermentation.

So far, it's on this fermentation stage that most of the attention in the cellulosic-ethanol field has focused. That's because although yeast naturally converts glucose to ethanol, there are no naturally occurring organisms that convert xylose and other five-carbon sugars to ethanol. *Escherichia coli* and other organisms do metabolize five-carbon sugars. But instead of making ethanol, they naturally produce a variety of acetic and lactic acids as fermentation products. To take advantage of the sugars that make up some 25% of plants, researchers needed to reengineer the workings of microbes.

The first to do so, in 1985, was microbiologist Lonnie Ingram of the University of Florida, Gainesville, who reported that he and his colleagues had inserted a pair of key sugar-fermenting genes into the bacterium *E. coli*. The genes redirected *E. coli*'s metabolism to convert 90% to 95% of the sugars in biomass to ethanol. Ingram's early *E. coli* strains weren't perfect. They could tolerate only about 4% ethanol in the final fermenting solution. Because the fuel must be distilled out of the surrounding water, a highly energy intensive process, ethanol makers strive to minimize the amount of distillation by using organisms that can tolerate the most ethanol possible. Since their early work, Ingram says he and his colleagues have managed to increase *E. coli*'s tolerance to about 6.4% ethanol. Ingram's strains have since been licensed to Celunol, which is building a 1.4-million-gallons-per-year cellulosic-ethanol plant in Jennings, Louisiana.

Other groups, meanwhile, have pushed to impart new talents to other organisms. In 1995, for example, researchers at NREL engineered a bacterium called *Zymomonas mobilis* to ferment xylose and other five-carbon sugars in addition to the six-carbon sugars it favors naturally. The work has since been taken up by researchers at DuPont in Wilmington, Delaware. And last year, DuPont's biofuels technology manager William Provine reported at the annual American Institute of Chemical Engineers meeting in San Francisco, California, that his group has recently come up with a *Zymomonas* strain capable of tolerating up to 10% ethanol. That process too is on the road to commercialization. Last month, officials at DuPont, Broin (a major corn-ethanol producer), and Novozymes announced that, as part of the DOE award, they will expand an existing corn-grain ethanol plant in Emmetsburg, Iowa, to produce approximately 30 million gallons of ethanol a year from corncobs and other cellulosic feedstock.

Yeast researchers have also gotten in on the act. Yeast is today's ethanol heavyweight, given its natural proclivity for turning glucose into ethanol. But because the microbe doesn't naturally process five-carbon sugars, researchers have expanded its abilities to it make better suited for more complex biomass feedstock. In 1993, researchers led by Nancy Ho, a microbiologist at Purdue University in West Lafayette, Indiana, spliced a trio of xylose-fermenting genes into yeast, making it the first yeast strain capable of fermenting xylose to ethanol. Since then, Ho's group has honed yeast's ability to convert a mixture of sugars to ethanol through improvements that include enabling it to use five-carbon

Company	Location	Size (Millions of gallons per year)	Feedstock	Completion date
Broin	Emmetsburg, Iowa	31	Corn stover (cobs and stalks)	2009
BlueFire Ethanol	Southern California	19	Waste wood	2009
Alico	La Belle, Florida	20	Wood, ag waste	2010
Abengoa Bioenergy	Colwich, Kansas	11.4	Corn stover, wheat straw, etc.	2011
Iogen Biorefinery	Shelley, Idaho	18	Ag waste	2010
Range Fuels	Soperton, Georgia	50	Waste wood, energy crops	2011

The winners. The U.S. Department of Energy recently backed six cellulosic-ethanol refineries.

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sugars other than xylose and boosting the speed at which the organism produces ethanol.

Tougher, softer, faster

Despite their successes in coaxing organisms to convert sugars to ethanol, most researchers recognize that much work remains to be done. "We are still climbing the mountain," McMillan says, and are "relatively low" on the slope. For example, yeast can convert a bath of glucose to ethanol in just a few hours, but microbes working on a complex mix of sugars can take 1 to 2 days to do the same thing. In a commercial plant, that means lower fuel output. So researchers around the globe are focusing heavily on increasing the expression of fermenting enzymes to step up the speed.

Another focal point for researchers, Ho and others say, has been toughening up the microbes. "All of these strains, while they are good at making ethanol, their robustness is nowhere near baker's yeast [working] on glucose," says McMillan. In addition to the intolerance many organisms have for ethanol, a wide variety of other compounds from broken-down biomass inhibit enzymes in fermentation.

Researchers are also looking for improvements in other parts of the process. One that has come under scrutiny is the chemical processing used to prepare plants for fermentation. Traditionally, researchers break apart the plant fibers by exposing biomass to dilute acids and steam. The result is a soup that can then be exposed to cellulase and hemicellulase enzymes, which further break fibers down into simple sugars for fermentation. But acid-steam processing has several drawbacks. For one, the acid reacts with sugars, reducing by about 10% the amount of total sugars that can later be fermented, MSU's Dale says. The acid byproducts, he adds, also inhibit celluloses and other key enzymes. Finally, the acids typically cannot be recovered and used again, which adds to the costs.

So Dale and other researchers are now commercializing a process that, instead of acids, uses basic compounds such as ammonia to accomplish the job. In recent years, Dale's group has developed a low-temperature process that readily breaks down leaves, grasses, and straws. It also allows facility operators to recover and reuse the ammonia and creates fewer enzyme inhibitors than do acid treatments. According to a recent analysis by Tim Eggeman, a chemical engineer with Neoterics International in Lakewood, Colorado, the technique could drop the cost of cellulosic ethanol 40 cents per gallon. At least for now, however, the technique doesn't work well with lignin-rich woody feedstock such as trees. So the hunt is still on for improvements in that arena.

Growth industry. A new agricultural-waste-to-ethanol plant in Jennings, Louisiana, is among the first of a new crop of cellulosic-ethanol facilities.

CREDIT: CELUNOL CORP.

A final target for many researchers lies inside plants themselves. Some companies and academic groups are working to reengineer plants such as corn, poplar trees, and switchgrass to boost their yields and make them easier to turn into fuel. In 1999, for example, researchers led by Vincent Chiang, a molecular biologist and organic chemist at North Carolina State University in Raleigh, reported that they had engineered poplar trees with 50% less lignin than conventional varieties and more cellulose instead. Originally, that work aimed at increasing the cellulose content for paper production. But Chiang says the result is equally valuable for improving the carbohydrates in trees for conversion to ethanol. "The idea is to generate as much polysaccharides as possible," Chiang says.

Since their early success, Chiang says, his group has been unable to reduce the lignin content below the initial 50%. More recently, he and his colleagues have turned to tinkering with genes that control the cellulose fibers within trees, aiming to reduce the crystallinity. Although the work is still unpublished, "we have altered several cellulose synthase genes and have pretty much figured out which are the important ones," Chiang says. The hope, he says, is to make it easier for cellulase enzymes to break down the polymer into glucose units during processing. That, in turn, would reduce the amount of enzymes that need to be added prior to fermentation and chip away at the overall cost. Related efforts are also under way to improve other potential energy crops—for example, reducing the lignin content and increasing the yield of grasses such as switchgrass and *Miscanthus*.

These and other advances lead alternative-fuel experts to predict that the cost of cellulosic ethanol will continue to decline, just the cost of as corn- and sugar cane– based ethanol has. "Each step has a newness to it that allows for optimization. Each one of them helps bring the cost down," says John Pierce, who oversees DuPont's bio-based technologies in Wilmington, Delaware. Although there are no commercial cellulosic-ethanol plants today, most estimates put the current cost of producing a gallon of cellulosic ethanol at between $3 and $4. By the time the full-scale production plants come on line beginning in 2009, that cost is expected to be about $2 a gallon. DOE's current goal is to drop the price to $1.07 a gallon, at which point it will be competitive with making ethanol from corn.

Yet even if cellulosic ethanol is destined to compete head-to-head with corn-based ethanol, it is benefiting right now by being in the second rank. "Corn ethanol has certainly paved the way for a lot of alternative fuels," says Ingram. In addition to pioneering the commercialization of enzymes used to digest starch and reducing their price dramatically, corn ethanol producers have created an infrastructure for handling large volumes of biomass and spurred gasoline suppliers to incorporate ethanol into their supply chains. Numerous U.S. automakers have also begun producing E85 vehicles, able to burn a mixture of 85% ethanol and 15% gasoline. Cellulosic-ethanol makers will inherit this established infrastructure, easing their way into the market—and perhaps even helping them create the first real alternative to gasoline.

–ROBERT F. SERVICE

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