

# Can biofuels finally take center stage?

Charlotte Schubert

**Today, ethanol and biodiesel are predominantly produced from corn kernels, sugarcane or soybean oil. But researchers and investors are increasingly upbeat about another biofuel feedstock, lignocellulose—the most abundant biological material on earth.**

**B**iodiesel and ethanol, despite their surging popularity as transportation fuels, barely put a dent in our use of oil. But that could change. The biggest well in biofuels has yet to be tapped—lignocellulose, the woody part of the plant and a potentially abundant source of energy.

Lignocellulose is everywhere—wheat straw, corn husks, prairie grass, discarded rice hulls or trees. The trick is getting the energy out of it. Right now that's an expensive process, limited to a handful of pilot plants. The race is on to optimize the technology that can produce biofuels from lignocellulose sources more efficiently—and biotech companies are in the running (Table 1). Already several companies and government-funded laboratories have engineered enzymes and microorganisms to optimize lignocellulose degradation and help turn it into fuel. And funding from both the public and private sectors is being ramped up (Box 1). The giant oil firm BP (London) announced in June that it would plow \$500 million into biofuel research in the next ten years.

In his State of the Union address in January, US President George W. Bush said that biofuels can help ease the “addiction” of the United States to foreign oil, and he proposed slating \$150 million for biofuels as part of his 2007 Advanced Energy Initiative. About 140 farm, forestry and environmental groups have endorsed the ‘25 by 25’ campaign, which advocates that 25% of US energy come from arable land by 2025. The EU has called for a threefold increase in biofuel use by 2010, to 5.75% of transportation fuel.

But it's still not clear whether the funding and incentives being offered by governments are sufficient to overcome the major

technical and economic hurdles to producing biofuels from lignocellulose. And if past oil crises are any indicator, political will may wither if the price of oil falls.

There's room for improvement in almost every facet of biofuel production from lignocellulose: cautious researchers say the enzymes are too slow, the microbes picky and the breed-

## Box 1 Show me the money

The US government's leadership and investment in bioenergy might trail countries like Brazil, Sweden, Austria and New Zealand, but American investors are by far the world's most bullish on biofuels. In just the last several months, Silicon Valley's legendary venture capital (VC) firm, Kleiner Perkins Caufield & Byers, launched a \$200-million fund for investing in bioenergy. Several other VC firms in California and Massachusetts have plans to raise funds later this year for financing bioenergy startups.

Wall Street is smitten, too, in large part because the share price of Pacific Ethanol (Fresno, CA, USA) has tripled in less than a year on the market giving the company a lavish market capitalization of close to \$700 million—despite having no operations. As *Nature Biotechnology* went to press, VeraSun Energy, America's second largest ethanol producer, along with Hawkeye Holdings and Aventine Renewable Energy were set to float an initial public offering (IPO), too.

Equities analysts argue that there has never been a better time to raise capital for bioenergy startups. Michael Judd, an ethanol analyst at Greenwich Consultants, told CNN last month that the spigot is wide open. “You couldn't find a better time to do an IPO because margins are probably at the highest they'll ever be,” he said. But Judd and others caution that making money in biofuels is not a straightforward proposition. Throughout the world, the main driver for bioenergy today are government rules mandating increased biodiesel and ethanol content in diesel and gasoline, respectively. Other drivers include bans on methyl tert butyl ether (a gasoline additive suspected of causing cancer), the high price of oil and the quest to gain energy independence.

The EU mandated that three times more than the current level of 2% of the total energy content of petrol and diesel needs to come from renewable fuels. Countries like Thailand are aiming for a 10% renewable mix in the next five years; India 20% by 2020. Sweden has stated that it aims to become 100% energy independent by 2020; most of this independence will come through its own nuclear power, but renewable fuels will likely make up the balance. According to the US Renewable Fuels Association, a trade association for the US ethanol industry, annual production totaled 3.9-billion gallons last year, up 15% from 2004. But estimates indicate that new plants to produce another 1.9-billion gallons a year are under construction and will come online by 2007.

All of this bodes well for biofuels. What could possibly go wrong? At present, less than 1% of the United States' fuel stations sell ethanol. Only a small fraction of the automobiles sold in America offer flex-fuel options. Until biofuel and flex-fuel vehicles can be purchased just as easily as the old-fashioned variety, bioenergy will be constrained, however.

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**Table 1 Selected biotech companies with biofuel programs**

Company Location	Focus	Funding/revenue
Agrivida Cambridge, MA	Engineering corn to produce enzymes for making ethanol post-harvest	Equity funded start-up with grants from the US Small Business Innovation Research Program, Mass. Tech Collaborative, and Renewable Energy Trust
Celunol Dedham, MA, USA	Has built pilot cellulosic ethanol facility, developing plans for a large-scale plant Engineers <i>Escherichia coli</i> to ferment all types of sugars released from cellulose to ethanol (licensed from U. Florida)	Equity-funded startup (formerly BC International)
Ceres Thousand Oaks, CA, USA	Developing energy crops (switchgrass) for cellulosic energy.	\$133.7-million licensing deal with Monsanto
Direvo Cologne, Germany	Improving cost-effectiveness of ethanol production from lignocellulose through directed evolution	\$19-million € in private funding
Diversa San Diego	Enzymes for converting cellulosic biomass to fermentable sugars, termite-derived enzymes in collaboration with DOE and Caltech; enzymes for increasing efficiency of biodiesel	Publicly traded on Nasdaq (market cap \$503 M) Participates in Integrated Corn-based Refinery Program Markets $\alpha$ -amylase for grain ethanol (by Valley Research)
Dyadic Jupiter, FL, USA	Proprietary platform for discovering, developing, and manufacturing novel genes from diverse organisms, in collaboration with Iowa Corn Promotion Board in DOE-funded program	Publicly traded on Nasdaq (market cap \$137 M)
Edenspace Systems Dulles, VA, USA	Developing corn, switchgrass and other crops as cellulosic ethanol feedstocks. Phytoremediation services	2005 revenues \$1.1 million \$750 K DOE grant Research alliances with NREL, USDA, Michigan State
Genencor (subsidiary of Danisco) Palo Alto, CA, USA	Protein and pathway engineering/Sells enzymes for pulp, fabric and other industries	\$17 million from NREL for low cost cellulases; 1.2 million € from French government for making ethanol from paper pulp
GreenFuel Technologies Cambridge, MA, USA	Algae bioreactor for recycling CO <sub>2</sub> and converting to high energy biomass	Partnership with NRG Energy (NY) \$6.8 million in Series B (Polaris, Waltham, MA, USA)
Iogen Ottawa, ON, Canada	Manufactures enzymes in paper, textile and animal feed. Has demonstration plant for converting biomass to cellulosic ethanol	\$130 million in private investment \$64 Canadian from Royal Dutch/Shell Group; \$30 million Goldman Sachs; \$15.6 from PetroCanada; \$10 million from Technology Partners Canada
Linnaeus Plant Sciences Vancouver, BC, Canada	Engineering plants to make oils that can replace petroleum products	Multi-year R&D agreement with Atofina (Paris)
Mascoma Cambridge, MA, USA	Biomass to cellulosic ethanol; designing cellulosic ethanol plant	\$4 million in private funding
Monsanto St. Louis	Crop analytics program for identifying corn with higher extractable starch soybeans with high oil and protein content	Publicly traded on New York Stock Exchange (NYSE) Market cap \$21 billion
Novozymes Bagsvaerd, Denmark	Producer of industrial enzymes and microbes; produces enzymes for reducing viscosity of plant mash	Publicly traded on Copenhagen Stock Exchange \$15 million from US DOE for low-cost cellulases
SweTree Umeå, Sweden	<i>Populus trichocarpa</i> expressed sequence tag and functional genomics program and fungi that dramatically increase the production of ethanol from agricultural and forest waste	Joint venture with intellectual property holder Woodheads (Umeå, Sweden). ~\$1 million in seed funding from Teknikbrostiftelsen (Umeå, Sweden) and ~\$2.2 million from Kempestiftelsen (Örnsköldsvik, Sweden)
Syngenta Basel	Developing corn expressing high levels of amylase-T	Publicly traded on NYSE Market cap \$13 billion
TMO Biotec Guilford UK	Developing thermophilic microorganisms to replace yeast in ethanol fermentation. Organism capable of fermenting both C5 and C6 sugars.	Partnering with six for share of €6-million research grant from EU

ing programs for specially tailored crops still in their early stages. Experts disagree about when facilities to convert lignocellulose to fuel will operate on an industrial scale—it may be five years or ten or twenty. But all agree that bringing down costs will be key.

### Back to the future

When Henry Ford built his first car the 'quadricycle' in 1896, he designed it to run on ethanol; in Germany, Rudolph Diesel built his engines to run on peanut oil. Although the first attempts at drilling 'rock oil' to supplant these biofuels met with skepticism, the consumption of petroleum inevitably surged with the rise of the automobile.

Over the past few decades, as fossil fuel use has continued to climb, researchers in academia and government laboratories have been working on converting biomass to fuel—with little attention or funding. Now, as oil prices begin to scale unprecedented heights, alternative fuels are again on the political agenda. Swarms of investors, consultants and press are buzzing around biofuel technology. Biofuels are fashionable again.

Worldwide production of biodiesel increased by 60% in 2005, and ethanol by 19% over the previous year's production, according to the Worldwatch Institute, a research organization in Washington, DC. Gas stations selling 85% ethanol blends are popping up throughout the

Midwest, US automakers are making specially tooled 'flex-fuel' cars to run on the same mixture and government incentives to produce and sell biofuels are locking into place worldwide (see Fig. 1).

A gallon of corn ethanol in the United States costs about \$1.00 to produce and contains about 70% the energy in a gallon of gasoline. Massive agricultural subsidies and a 51-cent ethanol tax credit keeps the fuel flowing. Some of the rise of bioethanol in political circles has been attributed to the intensive political lobbying of agribusiness giants like Archer Daniels Midland (ADM, Decatur, IL, USA), which has a vested interest in boosting ethanol to maintain corn subsidies. According to the Center for

Responsive Politics (<http://www.opensecrets.org>), agribusinesses, such as ADM, paid politicians a total of \$365 million between 1990 and 2005; in comparison, the oil and gas industry donated only \$182 million.

No one knows exactly how an expansion in the adoption of ethanol as an alternative fuel would transform arable land usage, influence fuel costs, or affect the economy at large. And it may not be the most environmentally friendly option. The US Environmental Protection Agency (EPA), for example, has acknowledged that high volatility of ethanol in gasoline blends under 10% ethanol can lower air quality and increase smog.

### From corn to lignocellulose

Most biofuels today come from the starchy and oily parts of plants. In the United States, corn grains yield much of the ethanol; in Brazil, it's sugarcane. Soybeans, canola seed, palm fruit and even hemp all can be used to make biodiesel (methyl/ethyl esters). But oils, seeds and fruits are not nearly as abundant as lignocellulose, more loosely dubbed 'biomass.'

Targets now promoted by the US Department of Energy (DOE) call for 30% of today's fuel use to be supplanted by 2030 with ethanol—60-billion gallons of it each year. Under that scenario, much of the fuel is slated to come from lignocellulose, which the DOE expects will become cheaper to make as the technology improves.

Researchers at the US National Renewable Energy Lab (NREL, Golden, Colorado) estimate the capital cost of a cellulosic biomass—converting facility which would yield 50-million gallons of ethanol per year, at \$215 million—about three- to fourfold more expensive than a corn-grain ethanol plant with the same yield. And once built, the ethanol from such a plant would cost about \$2.25 per gallon to produce. What's more, these estimates factor out much of the cost of pioneering a new type of technology.

Bruce Dale, a biofuel expert at Michigan State University (East Lansing), points out that when oil refining was maturing as a technology, it was not nearly as cheap as it is now. Converting lignocellulose to ethanol is estimated to consume 70% of the cost, and raw materials 30%, the exact opposite of oil refining today. "We have a lot of room to move to make our systems cost competitive," he said at the 28<sup>th</sup> Symposium on Biotechnology for Fuels and Chemicals in Nashville this April.

### The production process

People have been converting fruits and grains into ethanol via fermentation for thousands of years. Today, the process hasn't changed much. In ethanol production facilities, sugars

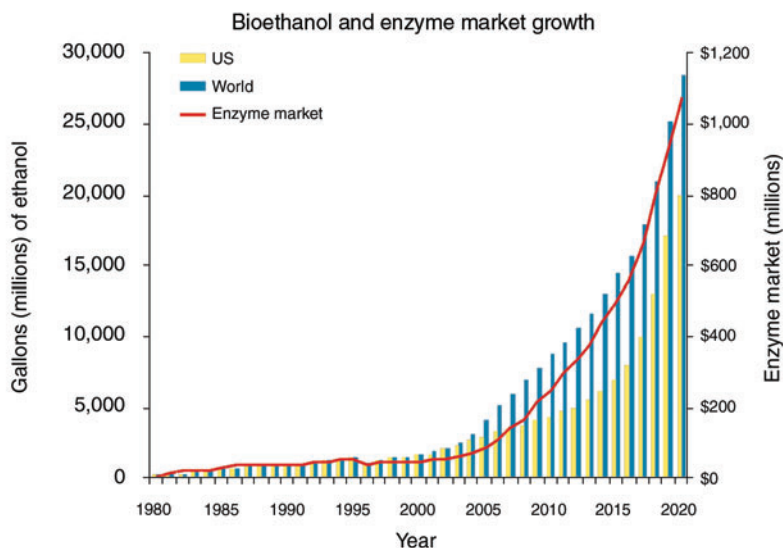


Figure 1 Rising production of biofuels. (Source: Renewable Fuels Association, Washington, DC)

and starches are turned into ethanol in a few simple steps. In the United States, where corn is the preferred feedstock, grains are processed with an enzyme that helps convert the starch into sugar, and then yeast ferments the sugar into ethanol (Fig. 2).

Whereas starch is soft, lignocellulose, the main component of the plant cell wall, has evolved to resist degradation. It consists of mostly hemicellulose and cellulose—glucose chains stacked into crystalline fibrils, largely impenetrable to water or enzymes. Lignin, a more complex macromolecule, makes up much of the rest. Wood, one potential source of lignocellulose, for exam-

ple, typically consists of 40–50% cellulose, 25% hemicelluloses and 25–30% lignins; the rest is made up of cell wall proteins and pectins.

One approach to extract fuel from lignocellulose borrows technology from the coal and oil industry to convert plant material into 'syngas,' mainly carbon monoxide and hydrogen. Syngas is then converted into ethanol or biodiesel by the Fischer-Tropsch process, invented in Germany in the early 1900s, usually using iron or cobalt catalysts. Another approach, popular in the United States, relies on enzymes and fermentation to produce cellulosic ethanol (Fig. 3).

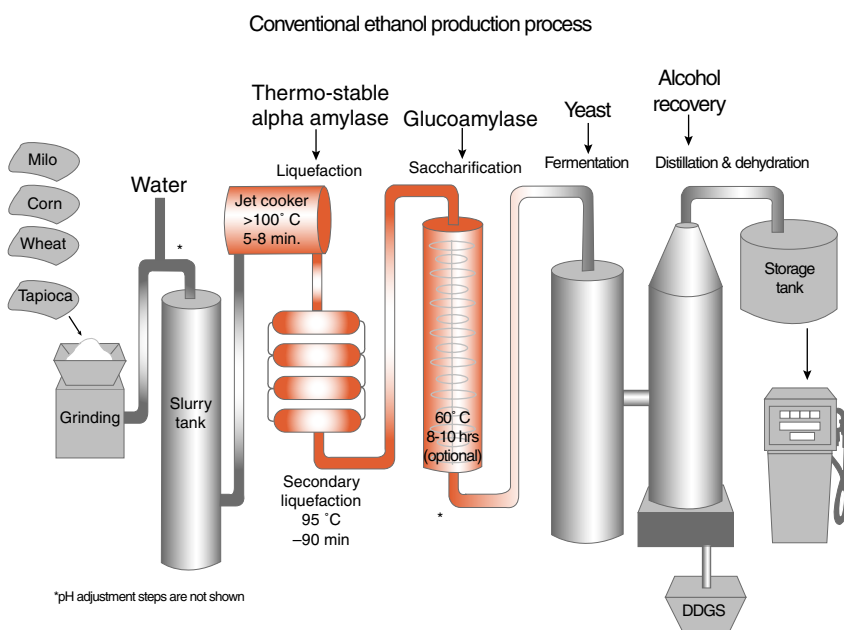
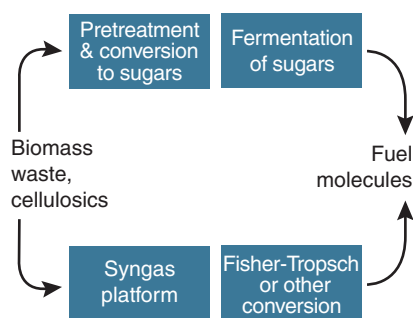


Figure 2 Steps in the process for making ethanol. DDGS, distilled dried grains with solubles. (Source: Genencor)



**Figure 3** The two processes for producing ethanol from biomass: biological conversion (top) and via syngas (bottom).

Only a few pilot plants have been built to produce cellulosic ethanol. The largest such plant is run by Iogen, an Ottawa-based private biotech company. The facility is designed to churn out about 260,000 gallons a year from 3,200 tons of straw. Like similar schemes, the Iogen facility is built around a multi-step process: a harsh pretreatment step, such as high temperature and acid, breaks apart the basic structure of lignocellulose, melting the crystals of cellulose. Then, enzymes convert the exposed cellulose into sugars. Finally, yeast is used to ferment the sugar into ethanol.

What's left is clumps of high-energy lignin, which is resistant to fermentation. Most designs call for lignin combustion to provide electricity to power the plant.

### Cellulases and strain development

To make the designs for cellulosic ethanol facilities more efficient, engineers are shaving costs from pretreatment and other steps. And biologists are focusing on two hurdles that have plagued the technology for decades—the high cost of cellulases, the enzymes that break down cellulose, and the limited ability of the microbes to ferment the breakdown products.

Iogen uses its own proprietary cellulases and is laying the plans for a 30-million-gallon-per-year facility with partners such as Royal Dutch/Shell Oil (London and The Hague) and Petro-Canada (Calgary, AB, Canada). Two other enzyme biotech companies, supported by large grants from the DOE, have brought down the costs of the enzymes. Genencor International (Palo Alto, CA, USA) and the Danish company Novozymes (Bagsvaerd, Denmark) have reduced the cost of cellulases about 20 fold to about 15–20 cents per gallon, according to NREL.

That's still expensive, according to Mike Himmel, a biochemist at NREL. By comparison, amylase, one of the enzymes that breaks down corn starch for fermentation, costs 2–4 cents per gallon.

Cellulases are less than ideal in other respects as well. “Cellulases do their job very slowly and at a time scale not very compatible with large-scale plants. They are probably good enough now to initiate demonstration plants but probably not good enough to meet these long-term goals,” says Himmel.

Skeptics are waiting to see if the claims for the Genencor and Novozyme enzymes hold up in larger facilities. Novozyme cellulases, for instance, will be used in a cellulosic ethanol plant being planned as an add-on to an existing Spanish ethanol facility by Abengoa Bioenergy a multinational ethanol company based in Seville, Spain.

Meanwhile, other researchers aim to overcome the limited diet of yeast. Yeast fermentation breaks down glucose and other hexoses but not the pentoses that result from the breakdown of hemicellulose, which comprises 15–50% of lignocellulose, depending on the type of plant. Lonnie Ingram, a University of Florida (Gainesville) microbiologist, has cre-

ated strains of *Escherichia coli* that break down both hexoses and pentoses into ethanol during fermentation, which he has licensed to Celunol Corporation (Dedham, MA, USA). He's also created an *E. coli* strain that can turn these sugars into organic acids used in plastics, which demonstrates the versatility of products that could be made from biomass.

Nancy Ho and colleagues at Purdue University (West Lafayette, IN, USA) have engineered a yeast strain to ferment both glucose and xylose, the main component of hemicellulose. They have licensed their yeast strain to Iogen and other companies.

Companies are tight lipped about improvements to their microbes, but some researchers caution such strains can suffer from drawbacks—such as susceptibility to inhibitors in bioreactors. Such problems can be ameliorated with changes in engineering and pretreatment processes, say Purdue researchers.

Meanwhile, some microbiologists dream even bigger—of the do-all microbe that chews up cel-

### Box 2 Fuels bloom from algae

Most biofuel producers focus on extracting as much energy as they can from terrestrial plants like corn and sugarcane, but a handful of researchers and entrepreneurs have their eye on an aquatic source—algae. Isaac Berzin once built bioreactors for experiments on the international space station, as an engineer at the Massachusetts Institute of Technology (MIT, Cambridge, MA, USA). But in his spare time he built another kind of reactor on the roof of the MIT power plant. The reactor captures greenhouse gas emissions like carbon dioxide from the power plant, and feeds them to algae growing in transparent tubes exposed to sunlight. The algae are then harvested, the oils extracted and turned into biodiesel.

Berzin is now the chief technology officer of GreenFuel Technologies (Cambridge, MA, USA), which is building a demonstration project in the sunny southwest United States this summer. The advantage of algae, says Berzin, is their sheer output. Single-celled algae can divide every few hours, much faster than any terrestrial crop. “Algae are the fastest growing plants on earth,” he says. “Algae are not bananas.” He claims his systems can produce 20,000 gallons of fuel per acre every year, about 40 times as much fuel as an acre of corn grain yields. Depending on how they are processed, algae could yield biodiesel, ethanol and other types of products.

Berzin is not alone in his enthusiasm. “We are pretty bullish on it,” says Eric Jarvis, a senior scientist at the National Renewable Energy Laboratory (Golden, CO, USA). NREL shut down its algae-to-fuels research program about ten years ago because of tight budgets and a move toward biomass. Now with high oil prices and interest in biofuels growing, “We are getting calls all the time from investors and energy companies. There is an incredible amount of interest.”

A few other companies have licensed similar technologies, including GreenShift, an energy company based in New York City. Jarvis thinks it is time for NREL to revive its algae program. He says there's lots more room for honing the designs of bioreactors, learning more about the algae and streamlining genetic engineering systems.

“It's an immature technology,” says Jarvis, “You are not going to put a plant on the ground and be profitable in a number of years.” But it's worth pursuing, says Jarvis. He notes that algae will not displace food crops, as with some biofuels. And they scrub power plant emissions. The MIT system captures 40% of the carbon dioxide output and 86% of the more powerful greenhouse gas, nitrous oxide.

Jarvis says he's impressed with Berzin's designs, but he is concerned about the capital costs. Berzin will not reveal the costs, but he notes that Greenfuel has raised \$20 million in venture capital, “This is not a charity effort,” he says. “They want to make money.” CS

lulose, ferments hexoses and pentoses, and spits out ethanol. That could reduce the number of steps required to produce cellulosic ethanol, and drive down the cost. But making such a bug is a complex task—cellulose-consuming bacteria are different than the yeast that ferment sugar into ethanol. Moreover, gene-splicing technology has only just emerged for some of the unusual microbes that degrade cellulose.

Undaunted, Lee Lynd at Dartmouth College (Hanover, NH, US) and his colleagues have engineered both yeast and a thermophilic bacterium to chew up cellulose and make ethanol, albeit at low yields and with some limitations—for instance the yeast still does not ferment pentoses. Lynd says he and others in the field could have an industry-ready microbe ready within a few years, but he concedes that other researchers are not so sanguine.

“The president says this out of the blue, and everyone says this is going to happen, but we haven’t really even set the table yet,” says Jared Leadbetter, a microbial ecologist at the California Institute of Technology (Pasadena, CA, USA). Leadbetter and Himmel emphasize the need for more basic research into lignocellulose, a substance scientists are only beginning to understand.

Himmel and other biochemists focus on the basic biochemistry of cellulases, with the aim of one day engineering one better than any they have seen in nature. Leadbetter searches for a better ‘tool chest’ of enzymes, turning to the world’s lignocellulose experts: wood chomping termites. To harvest his favorite species in Costa Rica, he has to dodge fragrant terpenes sprayed from a nozzle in their heads when they get angry. To get at the microbes in their guts, Leadbetter and his colleagues spend days chopping open the insects and extracting tiny drops of intestinal liquid.

After the termites chew wood, microbes in the gut break down the wood into acetate, which nourishes the insects. Leadbetter is now characterizing the ‘metagenome’ of the microbial community in partnership with Diversa (San Diego, CA, USA) and colleagues at the National Biodiversity Institute in Santo Domingo de Heredia, Costa Rica.

Leadbetter presented preliminary findings at a meeting sponsored by the Genomes-to-Life program at the DOE in February 2006. He has identified sequences for more than a hundred potentially useful enzymes that break down wood, including hemicellulases and enzymes that process pentoses. But getting them to work in a bioreactor or an engineered microbe is another challenge. “You probably need several dozen enzymes to degrade wood, and they all have to work effectively, stably and as rapidly as possible,” he says.

### Box 3 Biofuel cellulosic feedstocks

Most researchers envision that corn stover will be the first-generation cellulosic ethanol crop in the US—in part because of its massive acreage and to interface with existing ethanol producers. But few experts seem to regard corn stover as ideal, in part because it’s tough on the environment. Perennials such as poplar and switchgrass are less damaging. Compared with corn, switchgrass cultivation results in one-eighth the nitrogen runoff and one hundredth less soil erosion. The prairie grass has a big root system that adds carbon to the soil instead of depleting it. Birds also like to live in the stuff.

Switchgrass also has a lot of energy locked into it. Currently yields are about 4 tons per acre, and current cellulosic ethanol technologies produce yields of about 75 gallons per ton, says Dale. In the future, breeding programs could at least double switchgrass yields, and increase ethanol output to about 100 gallons per ton, says Michael Ladisch, director of the renewable resources engineering lab at Purdue University (West Lafayette, IN, USA).

But some researchers suggest avoiding a fixation on monocultures altogether. Studies on prairie grasses have found that they thrive when species are interplanted, interacting to provide nutrients to each other and use up every available ecological niche. David Tilman at the University of Minnesota and his colleagues found that plots with high plant diversity had 180% more biomass than monocultures such as switchgrass and they were better able to fend off insults such as drought<sup>3</sup>.

The key to high yields may be to find the right plant mixture, he suggests, “Once you plant these things they take care of themselves. I have seen prairie grass in Kansas that has been mowed for hay every single year for 100 years and it’s still highly productive,” says Tilman.

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#### Biology or chemistry

Some engineering companies and investors, particularly in Europe, are turning to another strategy. They are honing the technology for thermochemical conversion, which operates on the same engineering principles that turn coal into liquid fuel from syngas. But the process is still not cheap and easy. Biomass is messier than coal and gums up the facilities with tarry substances, reducing yield. The estimates for a full-scale facility range from \$300–400 million according to Jens Riese, who leads the industrial biotech group at McKinsey and Company (Munich), a management-consulting firm.

Riese admires scenarios that call for a set of interlocking facilities, hooked up to nearby cropland and transportation networks. With the profits from a conventional biodiesel plant, which costs \$20–30 million to build, smaller investors can finance a conventional or cellulosic ethanol plant, he says. And eventually they could add on a thermochemical facility to extract transportation fuel and other products out of the remaining lignin, or from all-purpose biomass.

“There are people who believe that syngas technology has stronger prospects than cellulosic ethanol conversion,” says Riese, pointing out it has advantages like easier adaptability to different types of plant material. Riese says it’s unclear which technology will prevail. “I think the jury is still out,” he says.

Other researchers hold out for even more fantastic technologies—for instance, bacteria that convert biomass into hydrogen, and algae that yield biodiesel (**Box 2**).

#### Bullish on biomass

Regardless of the technology or feedstocks (**Box 3**) used to produce biofuel, changes to crops and how they are grown has the potential to drive down the costs—for instance through increasing yields, growing them on marginal soil, or designing crops to melt more easily in a biofuel facility. Corn stover, the stalks and other inedible parts left on the field after harvest, could provide the test fuel for the earliest cellulosic ethanol facilities, say US researchers.

The reasons are both political and logistical. Big ethanol producers, such as Archer Daniels Midland, which produces 25–30% of the country’s ethanol, operate comfortably and profitably—and building facilities to convert biomass to ethanol currently carries huge financial risks. To reduce this risk, researchers at NREL envision cellulosic ethanol facilities plugged onto existing corn grain ethanol facilities, and their associated farming and transportation infrastructure. “The proponents of lignocellulose must work carefully with those who are involved in cornstarch production,” says Himmel, “We want this to be an effective alliance.”

But corn stover alone will not yield enough biomass to even come close to the ambitious DOE targets. And as Alex Farrell at the University of California, Berkeley puts it, “Corn is not the ideal crop”, in part because of its environmental footprint (**Box 3**).

Experts envision that dedicated biofuel crops will eventually become popular. These will be unique to a region’s climate and soil, such as the more environmentally friendly poplar and

switchgrass (a prairie perennial) or mixtures of species.

Several scenarios for cranking up the biomass output of the United States have been released, including the “Billion Ton” study in 2005 by the DOE and the US Department of Agriculture (USDA) (Box 4). The study concluded that biomass available for biofuels on US agriculture and forestlands could increase more than fourfold by 2030—reaching 1.3 billion tons, enough for more than 60 billion gallons of ethanol.

Corn stover is a part of that scenario, but the study also hinges on several assumptions about the future. These includes a doubling of the biomass yield of dedicated biofuel crops like switchgrass to about eight tons per acre (enough ethanol for 600 gallons with today’s conversion technology). That may seem ambitious, but many experts are optimistic.

“In some ways this is a conservative prediction,” says Brian Davison, director of bio-processing research at Oak Ridge National Laboratory (Oak Ridge, TN, USA). “We are not there now, but the assumptions sort of fall under the ‘research as usual should get us there’ category.”

Plant geneticists point out that during thousands of years of breeding, crops have not been selected for high biomass. So there’s still a lot of genetic variation available for breeders to work with. The first generation of biofuel feedstocks will emerge from conventional breeding or breeding assisted by molecular markers, say experts. At the recent Nashville meeting, for instance, Lawrence Smart and his colleagues at the State University of New York (Syracuse) reported that they had increased the biomass of shrub willow by about 40% after one generation of conventional breeding and selection.

In addition to agrochemical giants, such as Dupont (Wilmington, DE, USA), several biotech companies are also perfecting plants for biomass. In Nashville, researchers at Ceres (Thousand Oaks, CA) reported that they had identified an array of genes in *Arabidopsis thaliana* that boost biomass, increase nitrogen-use efficiency, and increase resistance to the stress of drought, cold or salt. Ceres has a \$137-million licensing agreement with Monsanto (St. Louis, MO, USA) to characterize genes for new varieties of traditional row crops like corn and soybean, and they have a molecular marker-based breeding program for switchgrass with the Samuel Roberts Noble Foundation (Ardmore, OK, USA), a nonprofit agricultural organization.

Some companies, including Ceres and Edenspace Systems (Dulles, VA, USA) are also engineering crops to produce their own inducible cellulases. Such plants, say researchers, could help overcome the cellulase problem plaguing the enzyme scientists. Plants with lower lignin content are also being developed, and they might also be easier to process in a cellulosic ethanol facility—but there is some concern that structural integrity could be compromised, ecological invasiveness could be increased, or soil microbe communities might be altered<sup>1</sup>.

In the near term, many plant biologists focus on more conventional biofuel goals—such as squeezing more oil from soybeans or sugar from sugarcane. Syngenta (Basel) is testing the regulatory waters for biofuels with a corn plant that could be processed more easily in a conventional corn grain ethanol facility. The corn produces its own amylase to accelerate the breakdown of starch and is queued up with regulatory agencies in the United States.

Crops like switchgrass will be grown in the same regions as native plants, increasing safety concerns about genes hopping species from genetically engineered plants. But plants engineered for lignocellulose-to-fuel conversion are years away, leaving time to develop technology to prevent cross breeding, says Nathanael Greene, a senior policy analyst at the National Resources Defense Council in New York City.

“We are long way off from where we as a society will need to tackle the GMO [genetically modified organism] crop issue for biofuels,” says Greene, “I think it will be a nonissue when it comes to that time.”

### Infrastructure

While biologists meddle with nature’s designs, engineers ready theirs. “The best way to learn is actually to go out and build and do,” said Andrew Richard in Nashville. Richard is a vice president of the SunOpta BioProcess Group (Toronto), which is providing components for a cellulosic ethanol facility in Spain with Abengoa. And these are just two companies drawing up blueprints.

Following a directive in the 2005 Energy Policy Act, the DOE has issued a call for proposals for up to three large-scale facilities that convert biomass to fuel or another useful products. The DOE will cover 40%, up to \$80 million, of the cost of a new plant—but it must be profitable and replicable. Researchers at NREL say that such incentives are necessary to help overcome the financial risks of pioneering new technology, but they emphasize the importance of getting it right; a big flop could put a chill on biomass.

Because of that concern, before the 2005 Energy Bill, the DOE had envisioned more

### Box 4 How ecofriendly are biofuels?

The acolytes of biofuels tout them as the ecologically conscious alternative to fossil fuel, and proclaim allegiance to the planet in boardroom conversations, hippie chatrooms and on bumper stickers. The fuels seem to fit nicely into the carbon cycle—being manufactured from plants, which suck up atmospheric carbon. But are biofuels really a guilt-free elixir to ease our environmental woes?

“Anyone who says that biofuels are greenhouse-gas neutral is wrong,” said biofuels expert Bruce Dale at the 28<sup>th</sup> Symposium on Biotechnology for Fuels and Chemicals this April in Nashville, Tennessee. Dale is professor of chemical engineering at Michigan State University (East Lansing). He goes on to say, “We will probably have to apply better management practices so they will be more greenhouse gas effective.”

The environmental benefits of biofuel hinges on its source, how the plants are farmed and harvested, how the fuel is extracted and how the fuel is finally blended. Numerous studies of corn grain ethanol, for instance, conclude that its greenhouse gas benefits are

marginal—but that’s with today’s way of manufacturing it. And if you switch the plant source from intensively farmed corn grain to biomass, the resulting fuel has high greenhouse gas benefits—and it takes less fossil fuel energy to produce. Such cellulosic ethanol also could leave a smaller footprint on the land, air and water—but that depends on how it’s made.

Researchers who conduct lifecycle studies examine inputs such as fertilizers and transport, and they stack that up against the energy contained in the resulting fuel. These analyses yield numbers for net energy gains and greenhouse gas emissions. Biofuels have the potential to put a dent in both: transportation comprises 23% of the world’s energy use, and emits 28% of greenhouse gases in the US, according to the Worldwatch Institute in Washington, DC and the Natural Resources Defense Council (NRDC), based in New York City.

Alex Farrell, an energy researcher at the University of California, Berkeley and his colleagues recently took stock of several lifecycle studies for corn grain ethanol<sup>2</sup>. Adding up the fossil energy inputs

## Box 4 continued

of petroleum, coal and natural gas resulted in 4 joules of energy produced from ethanol for every 3 joules of energy used<sup>2</sup>. That's a fairly slim net positive fossil energy ratio of 1.33. What's more, per mile driven, corn ethanol produces only 13% fewer greenhouse gas emissions than gasoline—and Farrell says there is great uncertainty in that number (Fig. 4).

The main reason for the poor greenhouse gas benefits of corn grain ethanol is the massive amount of nitrogen fertilizers applied to corn fields. These fertilizers are produced largely from natural gas, and are transformed by microbes into nitrous oxide, a powerful greenhouse gas. In addition, it's difficult to quantify this effect, which depends a lot on farming practices.

Although this and similar studies seem to tug the green label off of corn, some researchers have been trying to yank it off. David Pimental, an agriculture expert at Cornell University (Ithaca, NY, USA) and his colleagues are well known for their view that corn ethanol has a net negative fossil energy ratio and greenhouse gas contribution. Most other researchers dispute Pimental's conclusions, claiming he used outdated inputs and factors that others ignore—such as the energy consumed by farm laborers or the fuel used to make farming equipment and production facilities.

Pimental's conclusions clearly lie outside the norm of numerous studies by labs in academia government and nonprofit institutes. But even those who vehemently disagree with Pimental—and that is many others who conduct lifecycle analyses—admit that tweaking the numbers here and there with different inputs could tip the balance, “Almost every lifecycle analysis is missing something. When the differential is 30 or 40%, it's easy to play with the statistics to push the number to the positive or negative side,” says John Sheehan at NREL.

One particularly robust number for corn grain ethanol is the percent reduction in petroleum use, says Sheehan. Per mile driven, grain ethanol uses 80–90% less oil than gasoline, he says. That's because corn ethanol uses mainly coal and natural gas in its production. Critics note that this equation largely displaces one nonrenewable energy source with another, but ethanol boosters note that the tradeoff reduces petroleum consumption and reliance on foreign oil.

While the green tinge of bioethanol yellows, Willie Nelson and other advocates are singing the praises of biodiesel. The country singer has his own brand of biofuel, 'BioWillie' made from vegetable oil. Lifecycle studies give vegetable oil biodiesel a fossil energy ration of between 2 and 3. Estimates of greenhouse gas reductions also vary but cluster between 40–70% savings over conventional diesel per mile driven. That's because biodiesel requires less nonrenewable energy to manufacture than ethanol. And the most popular source of biodiesel in the US, soybeans, fixes its own nitrogen and so requires less fertilizer. But soybeans yield about a sixth as much fuel per acre as corn ethanol, according to Worldwatch.

“None of the fuels we have today are barely any good. Never mind ideal,” says Farrell. Farrell points out that lifecycle analyses fail to take into account many of the environmental drawbacks of biofuels such as soil erosion and water consumption. Seventy million acres in the US are devoted to corn—15% of which goes to ethanol production—and the agricultural runoff into the Mississippi River chokes off oxygen supplies and helps create a New Jersey-sized 'dead zone' in the middle of the Gulf of Mexico each summer.

Farrell is not sure cellulosic ethanol will be the ultimate fuel source—but it certainly wins out in the lifecycle assessments. Most fossil energy ratios for cellulosic ethanol range from about 4–10 and greenhouse gas reductions hover around 85%.

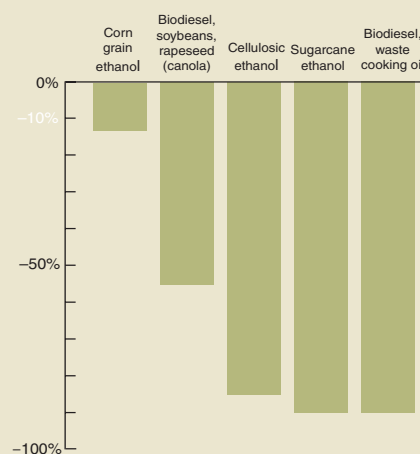
One major reason for the energy and greenhouse savings of cellulosic ethanol is that the lifecycle analyses factor in the energy savings of powering the ethanol plant with lignin, a byproduct of biomass-to-fuel conversion. Most designs call for this power source instead of the natural gas that fires most ethanol plants today. Without this factor, the energy savings of cellulosic ethanol drops substantially, but taking out lignin “totally misses the boat,” says Nathanael Greene, a policy analyst at the NRDC.

Another reason for the favorable lifecycle assessments of cellulosic ethanol is that biomass is expected to yield more ethanol per acre. Although corn yields vary greatly, Dale estimates that an average acre results in 430 gallons of grain ethanol; harvest half the stover, about the amount reasonable with current methods, convert that to ethanol and you would get an extra 150 gallons. High biomass crops like switchgrass also require less fossil fuel input than corn, upping the net energy yields.

Because cellulosic ethanol technology is not mature, researchers admit the lifecycle estimates are best guesses. For instance, Hosein Shapouri, an economist at the USDA says that he can obtain similar greenhouse and fuel savings if he assumes that waste biomass is burned to power existing ethanol facilities. On the other hand, scenarios factoring in improved technologies—such as higher biomass yields and more efficient ethanol manufacturing technology—can crank up the energy and greenhouse gas benefits even higher for cellulosic ethanol.

Whether cellulosic ethanol wins out in these and other measures of environmental impact depends on how the technology is implemented. “Cellulosic ethanol can contribute to our energy and environment goals if we do it correctly, and the issue is if we are going to do it correctly and how much land are we going to do it correctly on,” says Farrell.

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**Figure 4** Approximate greenhouse gas savings with biofuels. The percentage of greenhouse gas emissions displaced by driving a unit distance using biofuel instead of conventional fuel. Each crop is compared to the appropriate fuel: ethanol to gasoline; biodiesel to diesel. (Source: Worldwatch Institute and Argonne National Laboratory, reference 2)

modest plans to help underwrite a pilot project, according to Gene Petersen, a project manager at DOE. “We were not feeling there was enough maturity in the technology out there, but maybe we are being proven wrong,” says Petersen. Now he anticipates a solid lineup of innovative applications by the deadline in August—and like other researchers at DOE, awaits the final proposals before passing judgment on whether the plants are likely to be profitable.

With such projects, the US government contributes substantially more money for biomass R&D than the European Union and other countries, according to McKinsey’s Riese. In 2003, the EU spent only \$20 million, he says, whereas biomass budget at the DOE alone was \$90 million, and other agencies like the USDA also kick in.

President Bush’s \$150 million request for 2007 is a 60% increase over the 2006 DOE biomass and biofuels budget—although he’s also asked for cuts in other DOE budgets, such as industrial and automobile efficiency. And money earmarked for congressional pet projects ate up more than half of the DOE biomass budget last year—such as a \$1-million project in Ohio to make cement with the energy from burning discarded roofing shingles made from asphalt.

Biomass advocates say that even with the budget boost, the biomass budget is less than half that already authorized by Congress in the 2005 energy bill. They note that the speed at which new biofuel technologies take hold depends in part on government investment in research and market incentives.

An NRDC report forecasts that all of the US transportation needs for fuel could come from plants by 2050. But that scenario also depends on an unlikely boost in investment—\$1.1 billion in funding for biomass in the next six years, much of it for research to overcome the ‘recalcitrance’ of processing lignocellulose into ethanol. And the NRDC scenario also hinges on changes in community planning and big increases in automobile efficiency standards, “the cheapest and fastest way to end our addiction to oil,” says NRDC’s Greene.

## Box 5 Potential downsides

Biofuels are not necessarily a boon for the environment. In the United States, some newer corn grain ethanol facilities are switching to the use of cheaper coal instead of cleaner-burning natural gas as a power source. And this March, the EPA proposed allowing ethanol plants to more than double their emissions, a measure backed by more than 30 members of Congress.

Activists and policy makers in Europe are concerned that the European nations could ramp up imports of palm oil from tropical plantations to meet ambitious biofuel targets for their large diesel fleet. Although the fuel is plentiful and cheap, palm oil plantations are a prime driver of forest destruction in Southeast Asia, according to The World Wildlife Fund and other environmental groups. Palm oil acreage increased 11% last decade in Indonesia, according to Worldwatch, and plans underway would convert a million more acres to new plantations. Of course, using domestic European oils in fuels instead also could create new markets for palm oils in food.

In the United States, some policy makers are calling for importing sugarcane ethanol from Brazil, an option currently burdened by high tariffs. Sugarcane yields about twice as much ethanol as corn grain, and by most estimates has higher greenhouse gas benefits (Fig. 4), in part because it is easier to produce ethanol from it. But many sugarcane plantations are carved from the Cerrado, a biologically diverse grassy ecosystem in Brazil. All that ties into how we use the acreage we have. “That’s the \$64,000 question about biofuels,” says Farrell. “How much land?”

Ways to make room for biofuel crops include rotating them with food crops, using marginal acreage and gleaning biomass from conventional crops. In the United States, 349-million acres are devoted to agriculture, but 39-million acres of less productive land has been set aside—worldwide, 250-million to 2.5-billion acres of marginal land could be used for high biomass biofuel plants, according to Worldwatch.

A recent US government scenario says that over a billion tons of biomass could be made available within a few decades—enough for 60-billion gallons of ethanol. Under this scenario, 60-million acres would be planted with biofuel perennials like poplar and switchgrass. This study is more ambitious than one by the Rocky Mountain Institute, a think tank in Golden Colorado—and more conservative than one by the NRDC.

All the scenarios make assumptions about technology and agricultural changes in the future. The NRDC and US government studies both factor in big increases in the yields of switchgrass and a boost in the efficiency of extracting ethanol from it. And the NRDC study calls for supplanting all of today’s soybean crops with switchgrass, and then extracting protein for animal feed from the grass instead of soybeans.

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Even biofuel’s biggest boosters warn of the environmental impacts if biofuel technology is implemented carelessly (Box 5)—and they caution against viewing biomass as a panacea for our energy problems. “The thing to remember about fossil fuels is that they are the stored solar energy of eons and millennia that we are now burning over decades. We haven’t even leveled off our fossil fuel use yet,” says Leadbetter. “It’s

a very tall order to try and supplant that with another technology.”

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3. Tilman, D., Reich, P.B. & Knops, J.M.H. Biodiversity and ecosystem stability in a decade-long grassland experiment. *Nature* **441**, 629–632 (2006).