A field in ferment

To move US biofuels beyond subsidized corn will be a challenge, reports **Katharine Sanderson**.

Critics of the US ethanol industry have long derided it as an environmentally questionable subsidy to Mid-western farmers that simply serves a transparently political purpose. Voters in Iowa, the buckle in the US corn belt, get first say in the process of choosing presidential candidates. All such candidates are in favour of turning corn (maize), which the state produces in abundance, into ethanol. This pre-presidential support is good for the Iowan economy, but not necessarily that great for the environment.

Studies that compare the energy that goes into making ethanol — expended during the harvesting, fertilizing and transporting of the corn to refineries, and then refining it — with the energy that is released when it is burned routinely show that the net gain is at best small. The American Coalition for Ethanol says that ethanol contains twice the amount of energy that is used to make it; critics see no net gain whatsoever.

This criticism has had little effect, and since 1980, US ethanol production has risen from an average of 6,500 barrels (1 million litres) a day to 260,000 barrels a day. Federal mandates call for a further doubling by 2012. But it is increasingly clear to many in the industry that the criticisms of corn-based ethanol have merit, and in 2006, the need for an alternative was given the highest profile it could get when President George W. Bush brought it up in his state of the union address. In order to improve US energy security, he said, his government intended to make cellulosic ethanol (ethanol made from the rougher and woodier parts of plants) a competitive biofuel within six years.

IOGEN

Plant processing: Iogen's enzyme fermentor, a later stage of turning cellulosic feedstock into ethanol.

Corn stores

The advantage of an ear of corn as a source of ethanol (or for that matter as a bit of food) is that it is mainly starch, which is made up of sugars linked in a regular way with bonds that can be broken easily. Breaking the bonds between sugars and using yeast in the fermentation to produce ethanol is a straightforward task for the biorefineries. The disadvantage is that corn is a crop that needs a lot of inputs — fertilizers, water and pesticides — and that doesn't put as much of the sugar it creates through photosynthesis into its ears as one might wish. A lot of the sugar is instead turned into stalks and 'stover' — structural material rich in cellulose and considerably more difficult to break down.

Plants that store up a significant amount of energy in easily usable forms such as starch or sugar are exceptions, encouraged in their oddities by millennia of selective breeding — and of them all, only sugar cane grown in the tropics puts enough energy into its easily purified products to make bioethanol obviously attractive (see page 670). Most plants put the bulk of the energy they store up from the sun into cellulose and a related polymer, hemicellulose, and woody plants add another substance, lignin, to the mix. Cellulose makes up the plant's cell walls and, like starch, it is a polymer of sugars containing six carbon atoms linked one to the next. Hemicellulose, on the other hand, is based on a five-carbon sugar, xylose, although it contains many other sugars as well; its various components are thrown together in messy looking chains with many branches. Lignins are huge crosslinked jumbles of organic molecules which reinforce cellulose and hemicellulose to turn them into wood.

The energy that the plants put in to making the bonds in these various substances could, in principle, be extracted by fuel makers. And these molecules — particularly cellulose, which is both the most abundant and the easiest to dismantle — are much more plentiful than starches and sugars. But they are also much harder for microbes to break down; if they weren't, there'd be no trees, just pools of green goo. As yet, there are no cellulosic ethanol refineries operating at full commercial capacity, and assessments of the technology's readiness for market vary a great deal, as do opinions on how to get there from here. Government incentives and tax breaks might be one solution, but big energy companies also have a role to play, as do the smaller companies that have already worked on developing the technology, but have not yet found the best ways of spreading and licensing it.

The most expensive part of making ethanol from cellulose is pretreating the biomass to make it accessible to the enzymes that will then cut the sugars from the polymers so that they can be fermented. Typical pretreatments reduce the feedstock's volume chemically using acids, peroxides and ammonia, often along with some form of mechanical pressing or shredding. Unfortunately, this is not a step that can be skipped to cut costs, says Charles Wyman of the University of California, Riverside, because high sugar yields are essential, and untreated biomass gives very low yields. "The only step more expensive than pretreatment is no pre-treatment," he says. Instead, the hunt is on for pre-treatment technologies that involve fewer chemicals, require less energy and don't degrade the sugars that are set free in the process.

After the pre-treatment stage comes the snipping out of the sugars, which is the point at which biotechnologists think they can greatly improve on the current process. Abengoa Bioenergy of St Louis, Missouri, a subsidiary of the Spanish engineering group Abengoa, recently invested $10 million in Dyadic International, a biotechnology company that is concentrating on enzymes for degrading cellulose.

Based in Jupiter, Florida, Dyadic didn't start out as an energy company — in the 1970s it was a leading supplier of pumice for stone-washing jeans. But the enzymatic expertise it developed for distressing denim was then turned to a number of other ends. One of those was breaking down wood, a job that in nature largely falls to fungi. The company's research has centred on a filamentous mess of a fungus discovered by accident in a Russian forest that now, after ten years of processing and genetic engineering, makes up Dyadic's patented C1 fungal cell system. The fungus has been fully sequenced and encouraged to overexpress the genes that then make cellulases and xylanases — the proteins that break up cellulose and hemicellulose to produce fermentable sugars. "We have the world's most prolific filamentous fungus," boasts Dyadic's chief executive Mark Emalfarb.

Cellulose solutions

Emalfarb believes that the cellulosic ethanol market could eventually be worth $20 billion a year in the United States, and suggests that there is enough raw material available in the United States to produce 2.4 billion barrels of cellulosic ethanol a year. This is a bit more than half of what some estimates claim is needed to completely replace petrol as a fuel — the United States gets through some 3.3 billion barrels a year, but the energy content of ethanol is lower than that of petroleum.

The current leader in the cellulosic ethanol market, Iogen, also uses fungal enzymes. The company makes small commercial quantities of ethanol from straw at its pioneering cellulosic ethanol facility in Ottawa, Canada. As the first of its kind, this is an undoubted achievement. But

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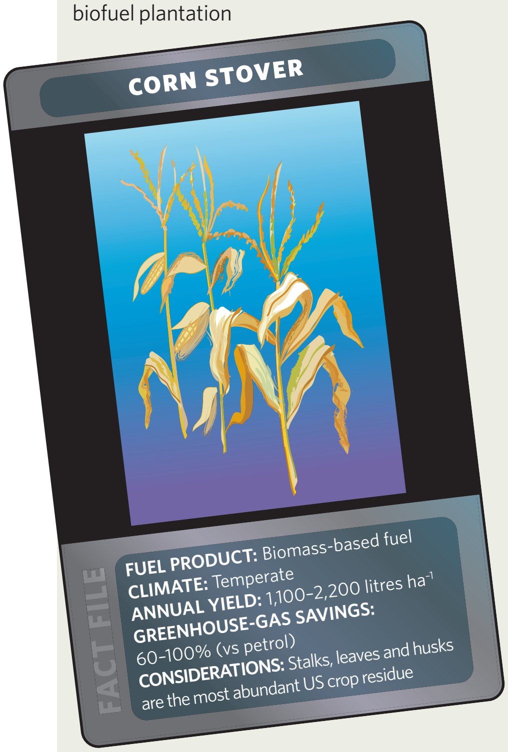
Prairie dreams

Endless hectares of grass, some tall, some short. Goldenrod, purple clovers and thistly, milky weeds, all growing together in all-but-virgin prairie. It doesn't sound much like a biofuel plantation — but some researchers say such mixtures could produce as much fuel as any alternative, and with a very low ecological impact.



"We have to find a way for people to see biomass as a renewable energy crop that won't compete with food or affect commodity prices," says David Tilman, an ecologist at the University of Minnesota in St Paul, and he thinks that mixed planting might be the way. Experimental plots containing mixtures of prairie plants are, perhaps unsurprisingly, more resistant to drought and pest than farmed monocultures[1]. Rather more surprisingly, they yield 2.7 times as much biomass on average[2], even outperforming the highly touted energy crop switchgrass. And in any cellulose-based system, biomass is king: the more there is of it — at least in theory — the more fuel can be extracted from it.

Tilman's prairies are one possible source of biomass, their appeal enhanced, he says, by the fact that the plants can thrive on land considered marginal for agricultural use. But they are not the only source under investigation. From improved strains of trees to plants genetically engineered for easy processing, there is a wide range of options, most of them with advocates who tout their ideas as enthusiastically as Tilman.

Richard Flavell, chief scientific officer of Ceres, a biotechnology company based in Thousand Oaks, California, gives full credit to Tilman's academic work — but says that how his ideas will play out in the context of biofuels remains to be seen. One issue is that a polyculture approach means finding the right mix of plants for lots of different conditions. Another is that, especially with technologies not yet fully developed, biofuel producers will prefer a uniform feedstock.

The enzymes that convert biomass into fuel are picky and easily inhibited by substances in various plants. That argues for dedicated plantations of predictable, well characterized plants — poplars, perhaps, in wetter climates, switchgrass or miscanthus, also known as elephant grass, in drier, warmer places. These perennial crops represent a significant improvement over corn as a feedstock: compared with corn, switchgrass cultivation requires less fertilizer and water, and results in one-eighth the nitrogen runoff and one hundredth the soil erosion, according to the even when it reaches its full capacity, which it is taking quite some time to do, it will be capable of producing only 2.5 million litres (16,000 barrels) a year, which is not a great deal.

Iogen chief executive Brian Foody is not worried. The critical steps for getting the right enzymes, the right pretreatment systems and the right yeast systems, have all been done, he says. "We just need to go through the nuts and bolts of the process." This means making sure that the demonstration plant works well enough to be replicated elsewhere — the company is looking to build new facilities in Idaho, Saskatchewan and Germany.

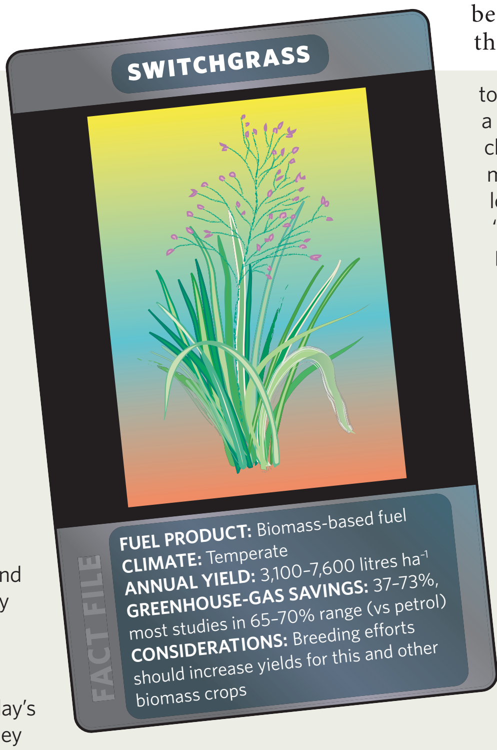
Iogen recently secured a $30-million investment from the bankers Goldman Sachs, bringing the total invested in it since the 1970s up to $130 million. But not all potential investors are convinced. "I don't really understand what Iogen is doing," says Matt Drinkwater, market analyst at New Energy Finance in London, UK. And his concerns are not unique to Iogen — many of the companies in the sector, he says, hold details of their processes so close to their chests that they are hard to evaluate, whether they be relatively small outfits such as Iogen or giants such as DuPont, which is also developing cellulosic ethanol technologies. Robert Wilder, who manages the Wilderhill clean energy index — the first such index to be accepted on Wall Street — agrees, but acknowledges the constraints that the chief executives of small cellulosic ethanol companies work under in terms of not tipping their hands to larger competitors.

Smells like green spirit

Perhaps because of these uncertainties over the technology's readiness, most of the money that has been invested recently in ethanol production both within the United States and beyond has been in the more traditional technologies. The sizable investments being made by agribusiness giant Archer Daniels Midland — the biggest ethanol producer in the United States and, perhaps tellingly, a company run by a chief executive who was recruited from the oil industry — seem mostly to be in traditional corn ethanol. The same applies to high-flying UK entrepreneur Richard Branson's recent investments in Ethanol Grain Processors of Tennessee and a new grain-based Californian ethanol venture, Cilion.

But there is some evidence that enthusiasm for investing in corn ethanol may be waning. Various ethanol companies that were riding high earlier in the year saw their stock slump after the summer when oil prices came down from their $78 a barrel peak.

This might mean the market is aware that, although subsidies may be able to keep it profitable for the time being, there is no way that corn ethanol can make a marked difference to long-term energy use in the United States. To make enough ethanol to start seriously displacing oil imports requires a process that can use cellulosic materials such as switchgrass, a tall prairie grass, or miscanthus, a grass imported from Asia, which provide far more tonnes of biomass per hectare than corn kernels ever can, and can be grown on land not suitable for conventional agriculture. Other sources could be farm waste or trees or newly engineered plants of some sort (see 'Prairie dreams'). This leads to something of an investing impasse: the companies in the business at the moment make money; the ones that might take it to the next stage do not, in large part because no one has made the heavy capital invest-



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US Department of Energy. And with current cellulosic technologies, switchgrass could yield roughly 4,000–6,000 litres per hectare, rivalling or beating the mature corn technology.

And that is before Flavell and his competitors get their hands on the stuff. Ceres wants both to enhance the biomass produced and to reduce the inputs needed, producing a crop that flourishes on marginal lands. The company's approach is to identify favourable genes in *Arabidopsis*, the lab rat of the plant world. So far, researchers at the company have found genes 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 to characterize such genes for new varieties of traditional row crops such as corn and soya bean. It is also using the genes in molecular-marker assisted breeding programmes for switchgrass and other crops in collaboration with the Samuel Roberts Noble Foundation based in Ardmore, Oklahoma.

Flavell points out that most biofuel crops have not been selected for high biomass, so breeders have a lot of genetic material to work with. The Noble foundation has already increased the yield of switchgrass by 25%, using conventional breeding.

But the genetic engineers are also laying plans, and looking beyond brute biomass to what could be seen as functionality — developing crops that actually help with the biofuel conversion process. For example, Ceres and other companies, including Edenspace Systems in Dulles, Virginia, are engineering crops to produce enzymes that would break down their own cellulose when triggered.

In the long run, there is no need to see polycultures and monocultures as mutually exclusive — both could have a role, and both could probably have their yields improved. If mixed feedstocks are not well adapted to today's finicky enzymes, they could still be used by 'thermochemical' systems such as those using the Fischer-Tropsch reaction (see page 677). And in time, the enzymes may catch up.

As Thomas Faust, biofuels research manager at the US National Renewable Energy Laboratory in Golden, Colorado, points out, the fundamental trade-off is between processing and environmental impact. "If you were going to genetically engineer a plant with desirable characteristics, a monoculture makes a lot of sense," he says. "From a conversion perspective, your ideal feedstock would be very homogeneous, a single grass, with constant moisture, available all year round. But to have a truly ecological, good-for-the-environment option, you would want mixtures." And according to Tilman, recent research on the biomass benefits of biodiversity[3] means that such mixtures could be found to suit many parts of the world: "The mechanisms behind the effects we are observing should apply to all other plant communities."

Charlotte Schubert

1. Tilman, D., Reich, P. B. & Knops, J. M. H. *Nature* **441,** 629–632 (2006).
2. Tilman, D., Reich, P. B., Knops, J., Wedin, D., Mielke, T. & Lehman, C. *Science* **294,** 843–845 (2001).
3. Van Ruijven, J. & Berendse, F. *Proc. Natl. Acad. Sci. USA* **102,** 695–700 (2005).

ments needed for plants that make use of the technologies that have already been piloted.

One way round this is to invest across the board. This is the strategy pursued by Vinod Khosla, the Silicon Valley venture capitalist who is one of the founders of Cilion. Khosla is also involved in cellulosic technologies through two companies based in Cambridge, Massachusetts: Celunol, which has just started to operate its own pilot plant, and Mascoma, which concentrates on process engineering and which last month raised $30 million in second-round venture funding. Farther afield in the biofuels world, Khosla is also a major investor in Kergy, a company that turns biomass into fuel in a completely different 'thermochemical' way, using just heat and catalysts. For some observers, such as Dan Schrag, a geochemist at Harvard University, these approaches are more attractive than fermentation, not least because they need no witches' brews made from fiddly feedstock-specific enzyme. "When the dust clears, cellulosic ethanol is unlikely to be where we end up," he predicts (see page 677).

To Drinkwater, investors such as Khosla, with their broad-based approach to the problem, are exactly what the industry needs to drive the market forwards and get it over the final bump it needs to clear before commercial success. Unfortunately, there are few such people. In their absence, many in the industry, not without self-interest, see the responsibility resting with governments to provide attractive tax incentives. "All forms of energy should face market prices that reflect the cost to society that they impose," says Foody. And to set those market prices, the right tax incentives and government mandates need to be in place.

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But government incentives won't make the scientists any smarter, and observers outside the pioneering companies believe there is still basic work to be done before those companies, or their eventual competitors, make the process economically viable. Thus they welcome increasing levels of basic research from the government, such as the US Department of Energy's pledge of $250 million to set up two bioenergy research centres that are largely focused on cellulosic ethanol. The European Union has set aside €100 million (US$132 million) for cellulosic ethanol in its seventh Framework Programme on research.

Ethanol alternative

Companies large enough to afford it are also following the basic research route rather than placing early bets on particular technologies. BP has announced it will invest $500 million over ten years to fund an Energy Biosciences Institute, which will be a dedicated facility based at a university. The University of Cambridge, Imperial College London, Massachusetts Institute of Technology, Stanford, the University of California, Berkeley, and Lawrence Berkeley National Laboratory have all been mentioned as possible hosts — the final decision is expected in December.

One intriguing possibility for such research to pursue is replacing ethanol with another form of alcohol. The fact that ethanol is easy to ferment can blind people to the fact that it has almost as many inherent problems as a fuel as corn has as a feedstock. Its tendency to pick up water wherever it goes makes it hard to transport, particularly in pipelines. It's corrosive. It's more volatile than one might wish. And its energy density is low compared with regular petrol.

For these reasons, BP and DuPont are working with British Sugar to adapt their ethanol fermentation facility in East Anglia to produce butanol — an alcohol with four carbons in it, as opposed to ethanol's two. This requires training microbes in new tricks, but it is not as hard a problem as breaking down woody plant material. The East Anglia plant will use locally grown sugar beet as the feedstock, but in the long term the aim would be to use a cellulosic feedstock. "We accept that taking stuff out of the food chain is not the right way to go," says Robert Wine, a BP spokesman.

Drinkwater thinks that an industry demand for butanol as an end product could actually increase interest in cellulosic approaches. "Most refiners would be much happier to use butanol than ethanol," he says. If oil companies become confident in biofuel technologies, investors would in turn be more confident of the biofuels industry as a whole, giving the industry that elusive final shove that it seems to need. ■

Katharine Sanderson writes on the physical sciences for *Nature*.

See Editorial, page 654.

T. GRAHAM/ALAMY

Pastures new: miscanthus, or elephant grass, is an alternative energy crop grown to produce ethanol.