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Lignocellulosic biomass, the woody parts of plants, can be enzymatically digested to yield chemical components that can be used to make biofuels.

LIGNOCELLULOSE

A chewy problem

The inedible parts of plants are feeding the next generation of biofuels. But extracting the energy-containing molecules is a challenging task.

BY KATHARINE SANDERSON

A potentially vast source of renewable energy sits just out of our grasp. Each year, more than 40 million tonnes of inedible plant material, including wheat stems, corn stover (the stalks and leaves) and wood shavings from logging, are produced — much of which is thrown away. Turning these discarded, woody bits of plants into 'second-generation' biofuels — which will not take food away from a hungry planet — has huge appeal.

The woody material that gives plants their rigidity and structure comprises three main types of carbon-based polymer — cellulose, hemicellulose and lignin — collectively called lignocellulosic biomass. When taken apart, these polymers yield chemical components that can be used to make biofuels. Cellulose, after all, is a polymer of glucose. And if this sugar can be extracted, it can be fermented to make ethanol or the longer-chain alcohol butanol. Hemicelluloses are polymers of various sizes that incorporate a range of different sugars, whereas lignin has a polymer backbone made from phenolic groups, which are ring-shaped, carbon-based structures. Other useful chemicals such as furans — molecules with a circular structure consisting of four carbon atoms and an oxygen atom — can be pulled out of lignocellulosic biomass and could serve as alternative, high-energy-density fuels. Most of the current efforts in second-generation biofuel production focus on ethanol, with furans and butanol at earlier stages of development.

The problem is that plants hold onto these chemicals with a tight grip. The glucose polymer chains in cellulose are largely insoluble and exist in crystalline microfibrils that make the sugars hard to reach (see 'From the field to the pump'). These cellulose microfibrils are attached to hemicellulose, which contains a variety of sugars, making it more complicated to convert to a single product such as ethanol. Surrounding all this is lignin, which protects the cellulose and hemicellulose. Lignin is a complex mess of polymers that are cross-linked to each other. The strong bonds that hold lignin's polymers together make it very difficult to break down. Added to that, the composition of lignin varies from plant to plant, and the true structure of this sturdy material remains unknown.

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At present, the best way to break apart these lignocellulosic materials and extract their chemicals for fuel production involves heat and strong chemicals. This is a complex process: once it has been mechanically ground up, the woody biomass requires pretreatment using heat, acid or ammonia to rip apart the lignin and expose the cellulose and hemicellulose inside. Enzymes can then penetrate the biomass and liberate the sugars, which are then fermented and distilled to produce ethanol. For now enzymatic biofuel remains at pilot scale. The enzymes so far discovered are not very efficient, to employ these techniques on a large scale is unsustainable — the existing process consumes more energy than is contained in the molecules it releases.

The good news is that there are alternative sources of enzymes. Some creatures happily survive on a diet of wood. So perhaps humans just need to find out how these enzymatic processes work and then apply them in an industrial setting.

THE TROUBLE WITH GRIBBLES

There are many potential sources of enzymes that digest wood. Some researchers are examining enzymes from the microbes found in termite guts or from wood-decomposing fungi that thrive on tree trunks. For example, biotechnology company Dyadic, based in Jupiter, Florida, has developed a designer fungus that it claims can inexpensively produce enzymes that rapidly digest lignocellulose. The enzyme technology developed by Dyadic is being used by Spanish energy company Abengoa in its Salamanca-based production facility, with a capacity of 5 million litres a year, to produce ethanol from corn stover and wheat straw.

At the University of York in the United Kingdom, plant cell-wall biologist Simon McQueen-Mason is investigating the marine wood borer *Limnoria quadripunctata*, also known as the gribble. These small crustaceans have long caused havoc in ports and harbours, eating away at moorings, piers and anything else made of wood. "This animal is very different from other animals that eat wood," says McQueen-Mason. "It has a gut devoid of microbial life." This means that the gribble — unlike the termite, which uses its intestinal bacteria to digest wood — must be able to secrete the enzymes needed to convert the wood into its constituent sugars.

McQueen-Mason and his colleagues are working to determine which genes allow gribbles to produce the correct mixture of cellulases — enzymes that can break down cellulose. So far, they have identified about 60 genes involved in production of glycosyl hydrolases, one type of cellulase. The gribble's cellulases seem to belong to three or four families, with each group working on a different part of cellulosic structure. It is not clear how gribbles deal with lignin, although a group of proteins called haemocyanins might be responsible. These enzymes are highly expressed in the gribble's digestive gland and have also been found in its gut, suggesting that they might be involved in digestion.

There is a lack of brave investors willing to take a gamble on a new technology.

It will take about three years for McQueen-Mason's team to fully investigate the gribble's digestive enzymes. In order to determine which enzymes are best at releasing sugars from unprocessed biomass, the researchers will work with enzyme firm Novozymes, headquartered in Bagsvaerd, Denmark. "It doesn't really matter where the enzymes come from, as long as we are able to produce them cheaply," says Novozyme's Claus Fuglsang.

Scaling up from the lab to industrial settings will not be simple, however. Digesting lignocellulosic material involves many enzymes for cleaving cellulose in different places. So, when a promising new enzyme is found, it must be tested in a range of enzyme mixtures to optimize the overall reaction. Improving the enzyme mixture is a priority says Fuglsang, who leads cellulosic ethanol research at Novozymes' laboratories in Davis, California: "With better enzymes comes the possibility of lowering chemical load and pretreatment."

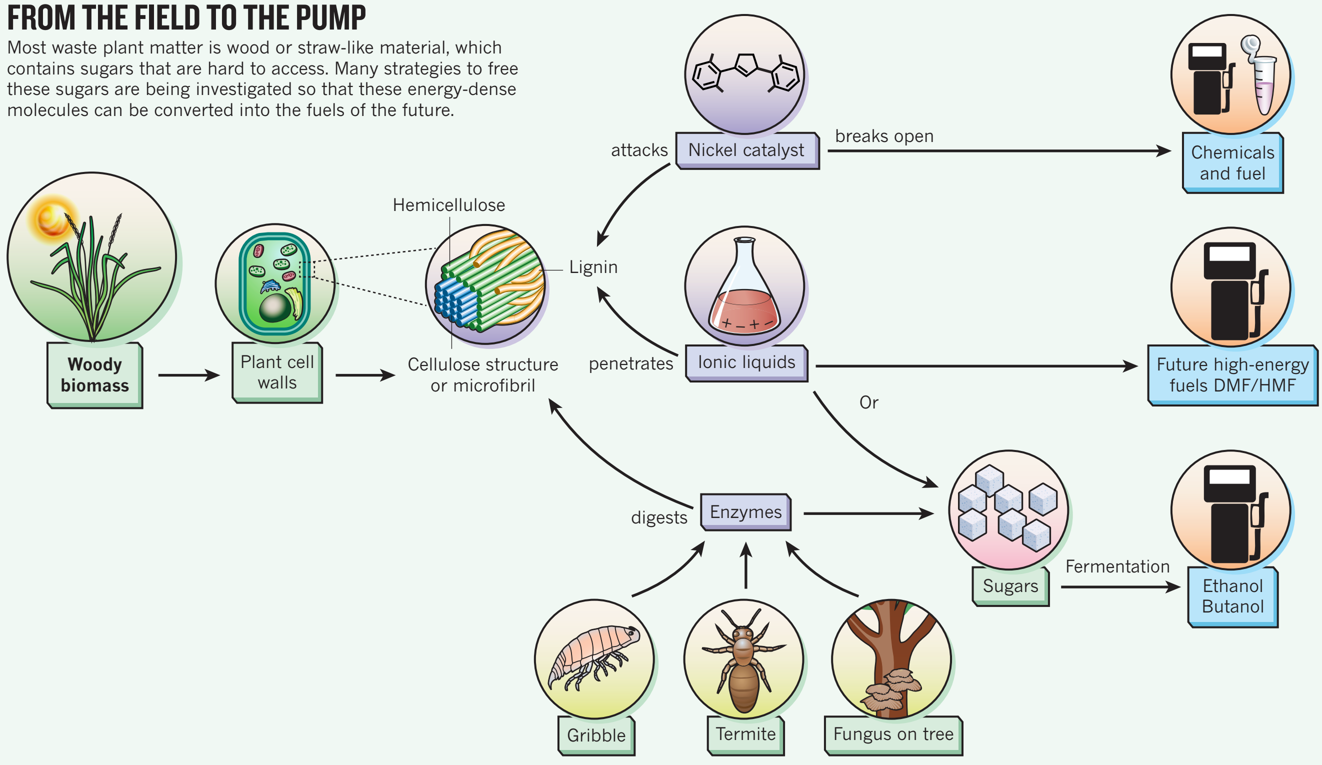
CHEMICAL SCALE

Chemical pretreatment processes are also in need of an overhaul. One weapon in the chemist's arsenal is a solvent called an ionic liquid. These are salts that become liquid at room temperature or just above, liberating their ions. They can penetrate lignin and liquify biomass, although exactly how is not yet fully understood. Chemist Brad Holmes and his colleagues at the Joint BioEnergy Institute in Emeryville, California, are using ionic liquids to dissolve crystalline cellulose and then reconstitute it as an easier-to-handle amorphous solid.

But the critical step in this process —

FROM THE FIELD TO THE PUMP

Most waste plant matter is wood or straw-like material, which contains sugars that are hard to access. Many strategies to free these sugars are being investigated so that these energy-dense molecules can be converted into the fuels of the future.



recovering the sugars from the ionic liquids — has proved challenging. Apart from the expense and chemical waste of losing some of the ionic liquid, there is another problem: if any ionic liquid is left in the sugar mixture, it prevents the enzymes from functioning in the subsequent fermentation step. Holmes' group is investigating boronic acid as a possible solution to getting the sugars out without destroying the ionic liquids, but the technology is still young. It probably will not be available on a large scale for ten years, he says.

Chemistry's role does not end with pretreatment. Processing plants for biofuels will need to be huge, requiring massive amounts of enzymes. Consequently, a purely chemical pathway to lignocellulosic biofuels may turn out to be simpler than an enzymatic route, says enzymologist-turned-chemist Ronald Raines, who works at the University of Wisconsin-Madison. "I have respect for enzymes, but they can be quite fragile," he says. "And they are certainly expensive." A chemical approach to the problem could take advantage of the accumulated wisdom of the chemical industry. "We know how to do things with chemistry on a very large scale," says Raines.

Raines' research group has developed a system that uses an ionic liquid consisting of N,N-dimethylacetamide and lithium chloride, which can dissolve cellulose without altering its chemical structure. This process can produce useful fuels from plant biomass in a single step at temperatures less than 140 °C (ref. 1).

The fuels that Raines' group are going after are from a different biofuel family from ethanol: the furans. With the help of a chromium catalyst, Raines can retrieve a chemical called 5-hydroxymethylfurfural, or HMF, which can, in turn, be used to make the promising petrol (gasoline) replacement fuel DMF, or 2,5-dimethylfuran. DMF can also be blended into fuel in much the same way as ethanol is now, and with around a 40% higher energy density than ethanol — a similar level to petrol — it has great potential as a biofuel. "We're very excited about DMF," he says.

Despite Raines' enthusiasm, DMF must undergo rigorous studies before it could be accepted as a biofuel. Safety is a huge concern, as DMF is known to be toxic to the nervous system, and the implications of DMF emissions aren't fully understood. Initial tests on DMF have been carried out and are promising: DMF has small-sized particulate matter emissions comparable to commercial petrol and a similar combustion performance[2].

In another approach, chemistry is also providing alternatives to the enzymes that serve as nature's catalysts. Chemists John Hartwig and Alexey Sergeev of the University of Illinois in Urbana-Champaign recently reported on a nickel catalyst that selectively removes oxygen atoms from lignin model compounds in precise places without destroying the entire molecule[3]. The less oxygen lignin has, the better it will work as a fuel.

The nickel catalyst works by breaking the carbon–oxygen bond that protrudes from related carbon-based alkyl ether, aryl ether and diaryl ether molecules, substituting a hydrogen atom for the oxygen but leaving the rest of the arene molecule undisturbed. Previous attempts to selectively break this carbon–oxygen bond have used high temperature and pressure, which resulted in the hydrogen atom attaching at other positions on the arene ring, creating a suboptimal and inefficient mixture of compounds. Using the nickel catalyst avoids this problem. However, the catalyst is currently in a soluble form, which makes it tricky to later separate the catalyst from the reaction products. Hartwig is working on developing a solid version of the catalyst so that liquids could flow over or through it, making separation much easier. The system also needs to be tested on naturally occurring lignin. In addition, to improve the sustainability credentials of this technology, Hartwig is looking for renewable or biomass sources for the hydrogen used in the reaction.

The gribble, *Limnoria quadripunctata*, secretes a digestive enzyme that wrecks wooden harbours, but might be useful in making biofuels.

SIMON CRAGG AND GRAHAM MALYON, UNIVERSITY OF PORTSMOUTH

Until these technologies become a reality, practical applications focus on enzymes and ethanol. In April this year, the chemical company Mossi & Ghisolfi, headquartered in Tortona, Italy, announced that it had started work on the world's largest cellulosic ethanol plant — a commercial facility in Crescentino, in northwestern Italy, that is set to be operational by 2012. The plant will have the capacity to make about 50 million litres of cellulosic ethanol a year using Novozymes' enzymes. To put this in perspective, it is twice as much as the 2011 target set by the US Environmental Protection Agency for the production of cellulosic ethanol for blending into fuel.

For chemical plants such as this one, there is more to consider when using new technologies than the nitty gritty of chewing up lignin or cellulose. Such issues are at the forefront of Katherine Smart's mind as the programme leader of LACE (lignocellulosic conversion to ethanol), an industry–academia collaboration based at the University of Nottingham, UK. In addition to research into topics such as the digestion of wheat straw, LACE investigates the economics and social and ethical issues raised by the introduction of cellulosic ethanol. LACE works closely with several industrial partners, including oil company BP, British Sugar and the Dutch life and material sciences company DSM, and is optimizing processes for producing ethanol from various woody wastes.

For lignocellulosic biofuel production, there are obviously questions about sourcing the required materials. For Smart, one of the biggest challenges is logistics: transporting the huge quantities of enzymes and biomass feedstock, or starter material, that are needed to the production plant. It might be necessary, for example, to incorporate on-site enzyme production. Fuglsang argues that the entire infrastructure for biomass-derived ethanol will be different from that currently used for fossil fuel distribution. He suggests that another solution is to build smaller production facilities near to the biomass in question.

The biggest hurdle faced by these second-generation biofuel technologies is not a dearth of clever science. It is the lack of brave investors willing to take a gamble on a new technology and the absence of enforced legislation that would encourage the production of cellulosic biofuels over first generation crop-based fuels. Until these problems are solved, woody plants will be able to cling tight to their stash of fuel for a little longer. ■

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