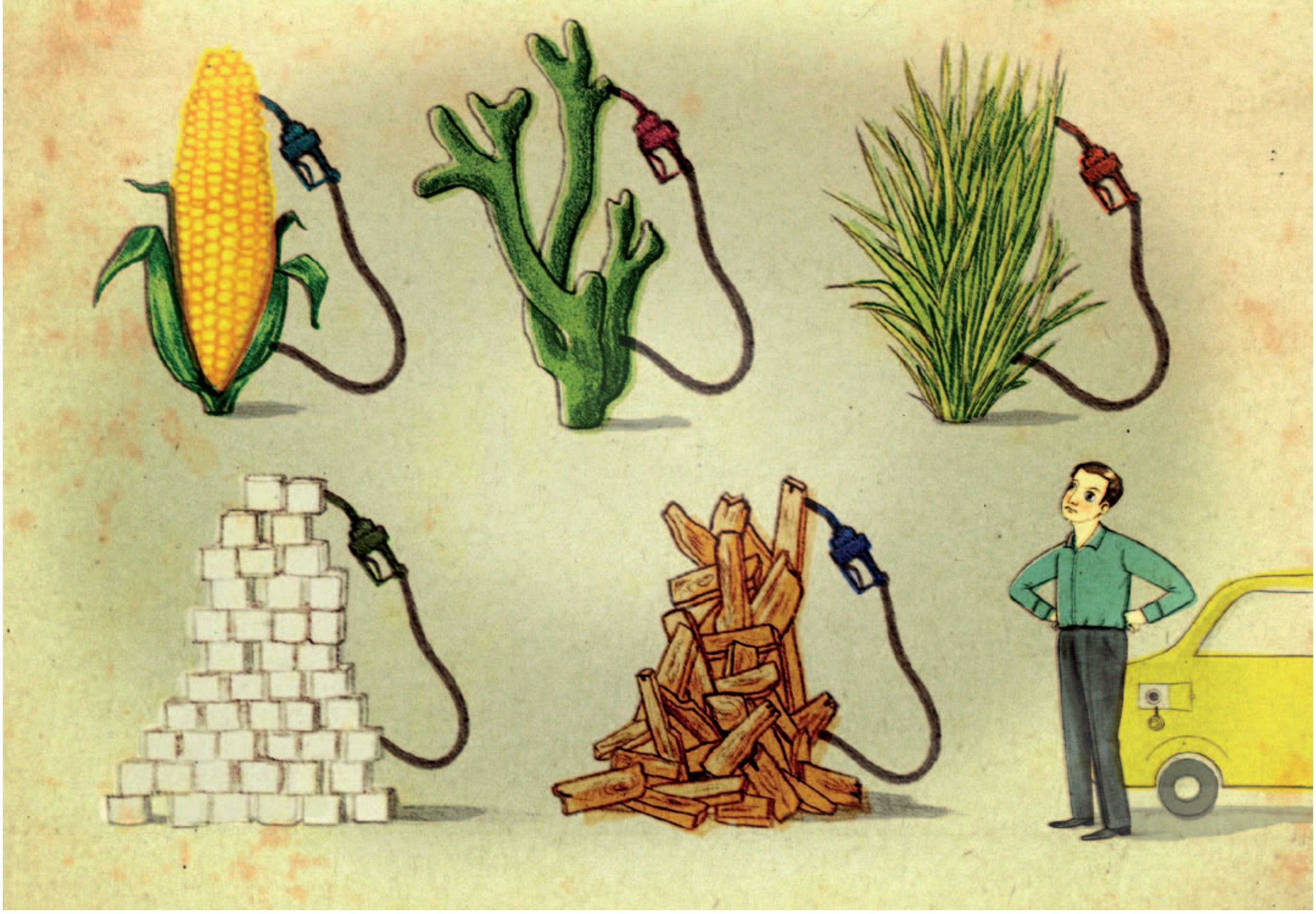

JONATHAN BURTON

FUEL OPTIONS

The ideal biofuel

A biomass-based fuel needs to be cheap and energy dense. Gasoline sets a high standard.

BY NEIL SAVAGE

A single word can sum up the biofuel of the future as envisioned by the people trying to create it — petrol.

"Our goal really is to come up with methods to make all the same molecules found in gasoline, jet fuel and diesel," says George Huber, a chemical engineer at the University of Massachusetts-Amherst, who is working on ways to turn plant organic matter, or biomass, into transport fuels. Like petrol (gasoline), an ideal biofuel should drop into today's infrastructure and carry enough juice to get any vehicle where it's going.

Ethanol, long the focus of the biofuel industry, doesn't meet those requirements. Compared with petroleum-based fuels, it's much less dense in energy: a litre of ethanol takes a car only about 70% as far as a litre of petrol, and ethanol cannot provide enough power for heavy trucks or aircraft. What's more, ethanol mixes with water from the environment, resulting in a more dilute fuel. It's also corrosive and so cannot easily be used in today's engines or be shipped cheaply through existing pipelines.

To overcome those limitations, researchers are trying to turn biomass into more complex alcohols than ethanol, as well as into hydrocarbons that more closely resemble those in petroleum, which is a mixture of different lengths of hydrocarbons. These scientists are developing processes — both biological and chemical— to produce substances that can be either placed directly in the fuel tank or slotted into the processing chain in existing refineries. And they want to use as much of the available biomass as possible, not just the simple carbohydrates that can be derived from sugarcane or kernels of corn, but also the harder to break down cellulose and lignin of corn stalks, wood and switchgrass (*Panicum virgatum*).

BIGGER ALCOHOLS

A 'higher alcohol' — a molecule with more atoms of carbon and hydrogen than ethanol — comes closer to the ideal. Ethanol has two carbon atoms linked to five hydrogen atoms, plus a hydroxyl group. The carbon–hydrogen bonds are where the useful energy is stored; breaking these bonds through combustion releases energy. Adding two more carbon atoms and four hydrogen atoms creates butanol, which is less corrosive and packs much more punch. With its four carbons, butanol has about 90% of the energy of petrol, which is a mixture of molecules with five to eight carbon atoms. Diesel and jet fuel consist of molecules with 9 to 16 carbon atoms.

Today, ethanol is often added to petrol to prevent pre-ignition, or "knocking." A move to higher alcohols could make alcohol-rich fuel blends more feasible. Today's petrol blends generally don't exceed 10% ethanol, but it's easy to imagine a fuel blend containing 50–70% of the higher energy, less corrosive alcohol butanol. Some proponents even say that unmodified car engines could work on butanol alone. "You can reach much higher levels of renewables in fuels if you go to these longer-chain molecules," says Michelle Chang, a chemist at the University of California, Berkeley who has induced microbes to produce butanol. Ultimately, butanol might also be cheaper to make than ethanol. When a batch of yeast produces ethanol from the sugar derived from, say, corn, the alcohol comes out mixed

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with water. The water has to be boiled off, which means using more energy to produce the fuel. But butanol and water don't mix, so they can be separated by less energy-intensive processes.

The problem lies in producing enough alcohol in the first place. Generating large volumes of ethanol is relatively easy. Yeast strains were domesticated for brewing alcoholic drinks thousands of years ago and are natural fermenters, turning plant-derived glucose into ethanol. Genetically manipulating them to make more ethanol is straightforward. Although there are organisms that naturally produce butanol and higher alcohols, most of them make those alcohols in tiny quantities, Chang says. "The question to ask is, how do you get the same yields that you get with ethanol. Nobody has matched those yields yet."

Chang recently found a way to boost butanol production by tenfold, at least in the laboratory. She plucked a combination of genes from different organisms and expressed them in *Escherichia coli*. Some of the genes are from a strain of the bacterium *Clostridium* that naturally produces butanol. The challenge, Chang says, is that *Clostridium* has its own agenda, geared more towards its survival than towards making large amounts of the alcohol. When the cell determines that there's too much butanol, the same enzymes that created the butanol start to break it down. Similar attempts by other researchers have therefore suffered from low productivity, yielding about half a gram of butanol per litre of glucose solution. So, instead of importing the entire butanol-making pathway of *Clostridium*, Chang mixed in genes of two other bacteria, *Treponema denticola* and *Ralstonia eutropha*. Those genes encode slightly different versions of the enzymes that control the fermentation — enzymes that are less likely to break down butanol.

This sort of manipulation is more difficult with longer carbon chains. The bigger the molecule, the more steps required to make it. And each step gives the cell the opportunity to divert production in a different, more natural direction. "To get good production, cells need to be healthy," says Shota Atsumi, a chemist at the University of California, Davis. "If we remove some pathways, many times, cells become sick." Atsumi has also inserted genes from other organisms into *E. coli* to induce the bacteria to produce various forms of butanol, as well as the five-carbon alcohol pentanol.

Atsumi's collaborator on the project — James Liao, a chemical and biomolecular engineer at the University of California, Los Angeles — recently produced a higher alcohol in a process that combines two sought-after advantages over ethanol production from corn. He started with a species of *Clostridium* that, unlike many butanol-producing strains, can digest cellulose, thereby enabling more of the available biomass to be used. Instead of pressing *E. coli* into service, Liao altered a pathway in *Clostridium* so that the bacterium produced the branched version of butanol — isobutanol. Isobutanol has the same chemical formula as butanol but a different structure that improves its engine performance and makes it easier to synthesize into other chemicals.

Several companies are trying to commercialize butanol or isobutanol production from biomass. In December 2010, the biotech company Green Biologics, of Abingdon, United Kingdom, announced a deal to provide its fermentation technology, based on a *Clostridium* strain, to two Chinese biochemical companies. Butamax Advanced Biofuels, a joint venture between the oil company BP and the chemical giant DuPont, has opened a demonstration plant in Hull, United Kingdom, and expects to have a commercial plant operating by 2013. And Gevo, an advanced biofuels company in Englewood, Colorado, is converting an ethanol production facility in Minnesota to produce about 68 million litres of isobutanol per year from 2012. Like ethanol, butanol would probably enter the market as a petrol blend.

Atsumi says that isobutanol yields need to increase by at least a couple of orders of magnitude to be economically viable. He doesn't see a need to coax microbes into making alcohols with longer carbon chains, because it's fairly easy to use conventional processes to convert isobutanol into other useful molecules. "Isobutanol is already a great biofuel," he says, adding that four- and five-carbon alcohols are "good enough for the fuel industry". Isobutanol can, for example, be dehydrated to form the hydrocarbon isobutene, which can in turn be used to make anything from petrol to jet fuel.

HYDROCARBON HEAVEN

No alcohol is likely to be the end point, however, if the aim is to fit into today's fuel storage and distribution system. "With alcohol fuel, and with ethanol in particular, you sacrifice a great deal of your gas mileage," says chemical engineer John Regalbuto of the University of Illinois at Chicago. Alcohol is "just not as energy dense as hydrocarbons," says Regalbuto, who is also a former director of the US National Science Foundation's catalysis and biocatalysis programme, which sponsors projects to convert biomass into fuel and other useful chemicals.

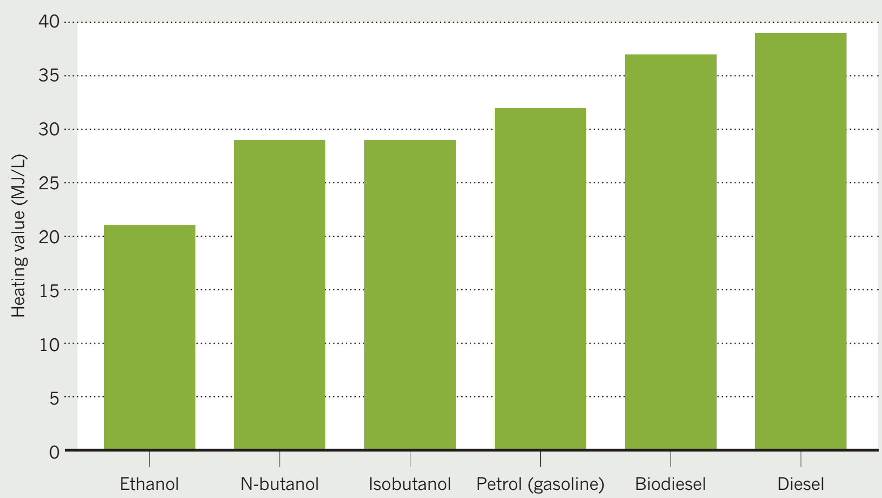
The closer the molecules that the microbes produce are to the molecules being burned in today's engines, Regalbuto says, the easier they'll fit into existing infrastructure. They'll be what biofuel experts call 'drop-in fuels'. Regalbuto likes to quote the motto of LS9, an industrial biotechnology company in South San Francisco, California, that is engineering microbes to produce chains of hydrocarbons: "The best replacement for petroleum is petroleum."

LS9 is one of several ventures using synthetic biology to redirect the fermentation process to produce hydrocarbons instead of alcohols. LS9's researchers identified genes that enable one kind of microbe, cyanobacteria, to naturally produce alkanes — one family of molecules consisting of only carbon and hydrogen atoms. The smallest alkanes (methane, propane and butane) are flammable gases, whereas petrol and diesel consist mainly of longer-chain alkanes. The company has been running a pilot-scale facility for more than two years and, in December 2010, announced it had raised US$30 million in investor funding to move towards commercial production.

Another way to get hydrocarbons from plant matter is to work with fatty oils, such as those produced by palm trees or soya. A chemical process called transesterification converts these oils to biodiesel. But because

PACKING HEAT

Biofuels vary in their energy density, but they all fall short of their petroleum-derived models.



HAN, L. ET AL. ANNU. REV. CHEM. BIOMOL. ENG. 1, 19–36 (2009).

Pyrolysis: cooking wood chips (left) above 400 °C for a few seconds with a catalyst (middle) produces a 'bio-oil' (right).

DR. JOUNGMO CHO

of the requirement for land to grow the crops, Regalbuto says biodiesel is unlikely to be produced on a large enough scale to meet fuel demand. Much work is being done to use algae to produce oil for converting to biodiesel, but progress has fallen short of the enthusiastic projections (see 'A scum solution', page S15).

Not every approach to producing hydrocarbons relies on microbes to digest biomass. James Dumesic, a chemical and biological engineer at the University of Wisconsin–Madison, derives fuel from plant matter through a multi-step chemical process. He turns biomass into a clear liquid called gamma-valerolactone, or GVL. Like ethanol, GVL can be blended into petrol. But GVL has an important advantage: it can be processed further, to become a hydrocarbon.

To produce GVL, Dumesic applies sulphuric acid to the cellulose in corn stover (the stalks and leaves left over after harvesting), sawgrass or wood. By contrast, during fermentation, enzymes are often added to biomass to break down the cellulose into the simpler sugar glucose, which the microbe can handle. "Here, we're going right past the sugar," says Dumesic, adding that sulphuric acid is far less expensive than enzymes.

This step produces equal amounts of formic acid and levulinic acid. Mixing a catalyst made of ruthenium and carbon into the levulinic acid transforms it into GVL, which contains 97% of the energy from the original biomass. Whereas fermentation requires several days to convert biomass, catalysis takes just "tens of minutes", Dumesic says. GVL can be shipped through existing pipelines or tanker trucks to a refinery for further processing. There, heating at high pressure in the presence of zeolite (an alumino-silicate catalyst commonly used in petroleum cracking) converts GVL to butene plus carbon dioxide. The butene molecules can be combined (with the help of another common catalyst) to yield longer hydrocarbon chains for diesel or jet fuel.

One problem with this process is that the sulphur in the acid tends to deactivate the carbon–ruthenium catalyst, a problem Dumesic has solved by using a ruthenium–rhenium catalyst. Now he's working on developing catalysts that use a cheaper metal than ruthenium.

Some researchers are trying to improve chemical methods, such as gasification and pyrolysis, that have long been in use for converting biomass to hydrocarbon-based fuels. Gasification, which dates back more than a century, involves heating carbon-containing materials to high temperatures in the presence of oxygen. The resulting syngas can be burned as fuel or converted to liquid fuel using the Fischer–Tropsch synthesis, a process developed in the 1920s. Pyrolysis works in a similar way: biomass is heated to between 400 °C and 600 °C for a few seconds, in the absence of oxygen, and then cooled rapidly to produce a liquid known as bio-oil. Bio-oil, analogous to crude oil, is a mixture of compounds that can be 'upgraded' to hydrocarbon-based fuels.

This upgrading involves adding a large amount of hydrogen to the carbon in the oil, which can cost more than the bio-oil itself, says Huber, a former student of Dumesic's. Huber has developed a pyrolysis process that, through the addition of a catalyst, doesn't stop at the bio-oil stage. The biomass is ground up and rapidly heated, and the resultant vapours flow through zeolites, which convert the vapours to benzene, toluene and xylene. These aromatic hydrocarbons can then be blended to yield a fuel that can be used in high-performance cars, for instance, as these require a high percentage of toluene. The whole process takes only minutes. "We think it's going to be significantly cheaper than gasification or fermentation," says Huber. The university has licensed his technology to the New York-based start-up company Anellotech, which Huber co-founded. "As long as we have a cheap feedstock, we can make our products at under $3 a gallon," says Huber. This April, gasoline prices were about US$4 per gallon in the United States (about US$1 per litre) and about US$2 per litre in the United Kingdom.

THE ONCE AND FUTURE KING

Huber doesn't foresee a single technology emerging as the king of biofuel processing. Instead, he says, there will be a mix that makes the best use of available resources and fits in with the various demands for fuels. "The future biorefinery is going to be like the petroleum refinery today," Huber predicts. "You're going to have a series of different units that all make different products."

But fuel will continue to be made of the same compounds that it is now. There's no reason to try to invent some new liquid, says George Church, a geneticist at Harvard Medical School in Boston, Massachusetts, because "alkanes are still a pretty good fuel". There's no better way to store energy for transport; petrol is "like a battery that's 50 or 100 times higher in energy density", says Church, whose synthetic biology research has contributed to LS9's technology and that of other biofuel companies.

Regalbuto is optimistic that biomass-derived, hydrocarbon-based fuel will soon slip seamlessly into everyday use. "I wouldn't be surprised if we're putting 'green gasoline' in our gas tank in five to seven years," he says. "And we won't even know it, because it will be a drop-in replacement." Longer term, he expects conventional cars, with their tanks of liquid fuel, will give way to battery-powered vehicles that depend on electricity generated from a mix of nuclear and renewable energy sources. Heavier vehicles — boats, aircraft, tanks and trucks — will rely on biofuel. Such a strategy, he says, could enable oil-dependent economies to end their reliance on imported petroleum. "Electricity for the light vehicles, biomass for the heavies, and we're energy independent in two decades," he says.

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Liao, who thinks the most promising feedstock will be algae, says a biofuel will be successful only if it can be made affordably and in large volume. "It has to be something that can be produced at the rate that we currently dig out oil from underground," he says. "Then we can talk about replacing petroleum." ■

Neil Savage *is a science writer based in Lowell, Massachusetts.*