

# Superfuel from superbugs

**There is a race to develop renewable, clean and carbon-friendly alternatives to fossil fuels. With a few exceptions, the initiative is with small start-up companies rather than the petrochemical giants. But these new companies are ambitious: quite simply, they want to replace today's 'big oil' by biofuels made by microbes like algae and bacteria.**

Oil is now well over \$100.00 a barrel; double what it was in 2006. Petrol and diesel prices in Europe and the US have risen accordingly. And many experts believe we have reached 'peak oil' – the point where worldwide oil reserves begin to fall.

So far, there is no credible alternative to fossil fuels. The idea of growing biofuel crops, which can be turned into petrol and diesel, is looking increasingly shaky. Diverting land away from food production is leading to food price increases and causing environmental damage as areas like Amazonia are cleared for biofuel production. In fact, the European Commission is having second thoughts about its insistence that 10% of road transport energy should be provided by biofuels by 2020.

Yet demand for petrol and diesel is increasing as the economies of the world's most populous countries, India and China, are booming. So where will tomorrow's oil come from? The answer, say the

chemists and biologists who are working with microbes, is by modifying nature: re-engineering simple life forms like algae and bacteria so they become tiny oil-producing factories.

The crude oil we currently pump out of rapidly decreasing underground reservoirs began as tiny aquatic organisms called plankton - including the various species of algae - which lived some 180 million years ago, about the time of the dinosaurs. When they died, the plankton sank to the seabed, where heat and pressure gradually turned them into oil.

## Oil from algae

Algae are simple organisms, which have colonised most areas of water – they are the 'scum', which floats on the surface of ponds and rivers. All algae have photosynthetic machinery, which converts energy from sunlight into chemical energy. The raw materials for this process are abundant: CO<sub>2</sub> and water. And, amongst the end products of algal photosynthesis,





are products called lipids, or oils, which can be extracted and processed into a variety of hydrocarbon fuels.

Basically, all algae biofuel research is aimed at finding recipes that will coax algae into producing more of the right kinds of lipids: by varying the amount of sunlight they receive; manipulating their nutrients and their growing conditions; and selecting and genetically-engineering different strains – which can then be turned into synthetic crude oil. In other words, they are trying to compress the work of hundreds of millions of years into a few days.

Researchers around the world are racing to turn algae into a viable source of oil products. Some varieties of algae are as much as 50% oil. That oil can be converted into fuel: biodiesel or jet fuel. But researchers need to find the right strains of algae and the most efficient ways of processing it. Converting algae oil into biodiesel uses the same processes that turn vegetable oils into biodiesel. So far, algae oil has been processed in the laboratory. Commercial demonstration plants will be

constructed within the next two years. But will it be economical? No-one knows yet.

Algae are amongst the fastest growing plants on Earth. They can double in size in a matter of hours. In some species, oil accounts for half their body mass. Whereas a hectare of corn harvested for biofuel can produce about 30 litres of oil a year, a hectare of algae could produce nearer to 30,000 litres..

Another potential benefit is that specific carbon structures found in different strains of algae may allow the development of 'designer fuels' tailored to specific uses: jet fuel, for example. And, since algae take up CO<sub>2</sub> from the atmosphere, the process can be used to reduce carbon emissions.

Algae are hardy, single-celled organisms found all over the planet, from the Antarctic



to sewage ponds and salt lakes. Algae do not need much to survive: just sunlight, a culture medium, and air. In fact, they thrive on air with a high CO<sub>2</sub> content. There are more than 10,000 known species of algae. But just two species are proving to be most popular in biofuel research: chlorella and cyanobacteria. Chlorella is a standard organism which grows quickly. A lot is known about it and it is easy to handle.

### Farming algae

Algae farms are nothing new. The Japanese were harvesting algae for food from open ponds centuries ago. But there are difficulties with open ponds: for example, sunlight doesn't penetrate more than a few centimetres below the surface and wild algal strains can contaminate the ponds. So algae biofuel researchers are designing closed systems called 'photobioreactors' to provide the right mixture of sunlight and nutrients while keeping wild algal strains out.

GreenFuel Technologies Corp., a Massachusetts startup, is developing algae technology specifically for removing CO<sub>2</sub> from power station emissions. Instead of venting CO<sub>2</sub> into the atmosphere, power station exhaust is bubbled through bioreactors

containing algae and seawater. During photosynthesis, the algae 'eat' CO<sub>2</sub> and other pollutants like nitrogen oxide. During tests, the technology has reduced power station CO<sub>2</sub> by half, even during cloudy days when little sunlight is available. When harvested, the algae can be dried and used as fertiliser and animal feed as well as being turned into biofuel.

GreenFuel Technologies are now looking at ways of tailoring algae to fit specific power stations. The idea is to gradually 'train' algae species in the laboratory to adapt to the flue gases they will be feeding off in their new habitat: "We just use the natural tendency of algae to adapt to any environment," says Isaac Berzin, GreenFuel's founder. Berzin believes that algae technology is particularly suited to cleaning up power station emissions: "This technology needs CO<sub>2</sub>. CO<sub>2</sub> is not an issue. You're located next to a CO<sub>2</sub> generating facility. The technology is exciting because it turns pollution into green fuel."

GreenFuel has already tested two prototype systems, and plans to build several pilot plants at power stations across the US. GreenFuel claims that a hectare of bioreactors could produce 30,000 litres of biodiesel every year as well as 9,000 litres of the petrol substitute ethanol.





### The light factor

It's not just American companies who are involved. Subitec GmbH, a spinoff from the Fraunhofer Institute, Germany, has constructed a modular algae bioreactor. Called a 'flat plate airlift reactor' or FPA, it is a vertical reactor made from transparent plastic, which contains about 30 litres of liquid culture medium. FPAs are closed systems which can be kept sterile and, more importantly, can ensure that lighting conditions are regulated so that the algae can thrive. Controlling lighting is one of the major problems in algal technology: it has proved difficult to design large-scale bioreactors,

which use light energy efficiently.

Subitec claim to have solved this problem, and are building a pilot plant of nearly 300 modules. Like GreenFuel, Subitec believes it would be best to site algae farms next to power stations, so they can feed CO<sub>2</sub> from burning fossil fuels to the algae. Subitec claim their FPA modules are ten to fifteen times more efficient at taking up CO<sub>2</sub> than an equivalent area of forest.

### Diesel fuel made from algae

One of the few companies to have actually road-tested algae biodiesel is Solazyme, Inc., a San Francisco-based research laboratory

started by two college friends. The biodiesel even has a trade-marked name – Soladiesel – and it has been powering a factory-standard Mercedes engine around the West Coast of America for several thousand miles. Soladiesel, says Solazyme, is non-toxic, biodegradable and reduces greenhouse gas emissions: in other words, a 'superfuel'.

Solazyme, started in 2003 by Jonathan Wolfson and Harrison Dillon, is a synthetic biology company turned oil producer. Its laboratory has produced thousands of gallons of biodiesel by using a radically different way of coaxing algae into producing oil.

Where most biofuel companies are looking

at ways of maximising the amount of sunlight that reaches algae, Solazyme is avoiding it: in fact, its researchers keep their algae in the dark. Without sunlight, their ability to photosynthesise is restricted. Instead, other metabolic pathways that produce oil become active, resulting in much higher yields. Because they are denied sunlight, the algae need another energy source: sugar.

"The algae don't produce oil through a direct sunlight process," says Solazyme's CEO, Jonathan Wolfson. "Instead, we feed sugar to the algae. They're a thousand times more productive at making oil when you feed them sugar than feeding them sunlight."

Harrison Dillon says the sugar currently comes from sugar cane, of which there is a world surplus. But it could come from any form of cellulose that is easily and cheaply available. Algae are extremely efficient at processing sugars from cellulose. In fact, they thrive on rotting plant life. So they could be fed on plant waste, grass, or wood chips. Such sources of cellulose, says Dillon, require less energy, water and land to grow than traditional biofuel sources like corn grain, so there's less impact on the

environment.

Solazyme's researchers have put a lot of effort into genetically-modifying algae so that they produce different types of oil that can be extracted and processed into fuels other than diesel – jet fuel, for example. And they are also modifying their metabolic pathways so that they become more productive:

"We take strains of algae and we optimise them for maximum oil productivity," says Wolfson.

### Bacteria factories

Not all biofuel researchers are working with



algae. Instead, they are looking at their close relatives, bacteria. Photosynthetic bacteria like cyanobacteria are so close to algae they are often called 'blue-green algae', and they also have the metabolic pathways that produce lipids which can be turned into biodiesel. But some researchers are attempting to re-engineer an unlikely candidate: *E.coli*, more commonly associated with food poisoning in humans.

George Church, Professor of Genetics at Harvard Medical School, was one of the cofounders of the Human Genome Project, and a prodigious inventor of analytical tools in genetics research, including DNA synthesising and sequencing. Church is also a pioneer of synthetic biology, in which the genomes of existing organisms are redesigned so that they produce useful products like pharmaceuticals. But Church also believes that bacteria can be engineered to directly produce diesel and petrol. In other words, instead of pumping oil from the ground and processing it into petroleum products, a whole new industry would brew petrol and diesel in the same way we brew beer. Since bacterial petroleum products would be the same as today's fuels, the same infrastructure of fuel depots, tankers and petrol pumps could be used. Engines wouldn't need to be modified: the only difference would be that the petrol at your local garage was produced by bacteria.

Church's research has led to a startup company, LS9, which has already trademarked the name 'renewable

petroleum'. LS9 says this can be made by genetically-engineering various bacteria, including *E.coli*, to produce hydrocarbon chains that are indistinguishable from – or even better than – natural hydrocarbon chains.

Church's company uses a combination of techniques to modify the genetic pathways that bacteria use to make fatty acids, one of the main ways bacteria store energy.



Fatty acids are essentially hydrocarbons and acid - remove the acid, and the resulting hydrocarbons can be turned into fuel. LS9's researchers are using two techniques: redesigning genes on a computer and synthesising them; and using genetic engineering to insert new genes into the bacteria. In both cases, the resulting modified bacteria manufacture and excrete hydrocarbon molecules to order. LS9 claims it can make oil that is actually superior and cleaner than natural crude oil. And a major economic benefit is that synthetic crude oil could be sent direct to a standard refinery to be processed into diesel, petrol or any other petrochemical product. So, unlike hydrogen or ethanol, energy companies would not need to invest in new storage and distribution systems: "Instead of having

the infrastructure adapt to biology," says Church, "we're using biology to adapt to the infrastructure."

### Synthetic biology

Jay Keasling, Prof of Chemical Engineering at the University of California, Berkeley, is one of the world's leading practitioners of synthetic biology or 'bioengineering', which regards living organisms as assemblies of parts that can be modified, redesigned or even built from scratch from standard biological parts like proteins and genes:

"We think of biological components as parts you assemble and try to get to function as a whole," says Keasling, whose San Francisco company, Amyris Biotechnologies, is one of the first to take synthetic biology into the marketplace. Within a year, the company hopes to have brought the technology to cheaply mass-produce an antimalarial drug called artemisinin to the stage where it can be handed over to a pharmaceutical company.

Amyris and Keasling's first project was supposed to be algae-produced biodiesel.

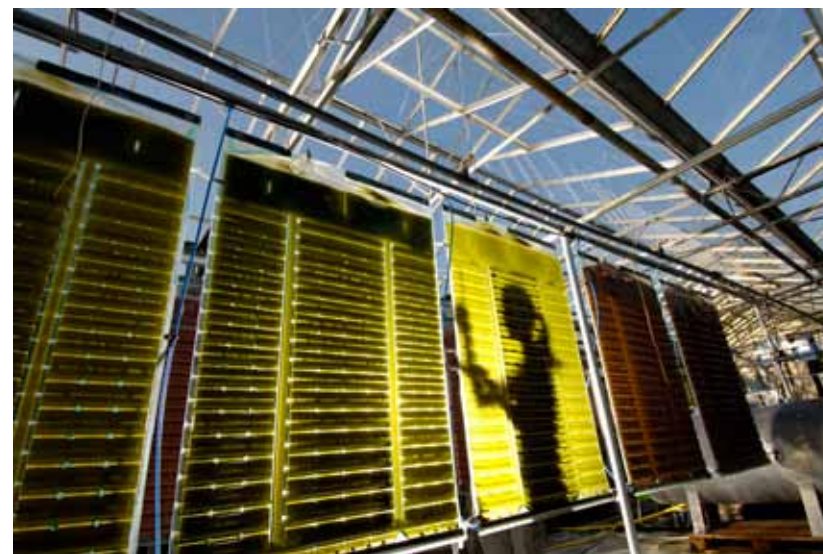
But Keasling had already begun research on synthetic artemisinin, which was then funded by several millions of dollars from the Bill & Melinda Gates Foundation, giving the company the resources to develop the antimalarial.

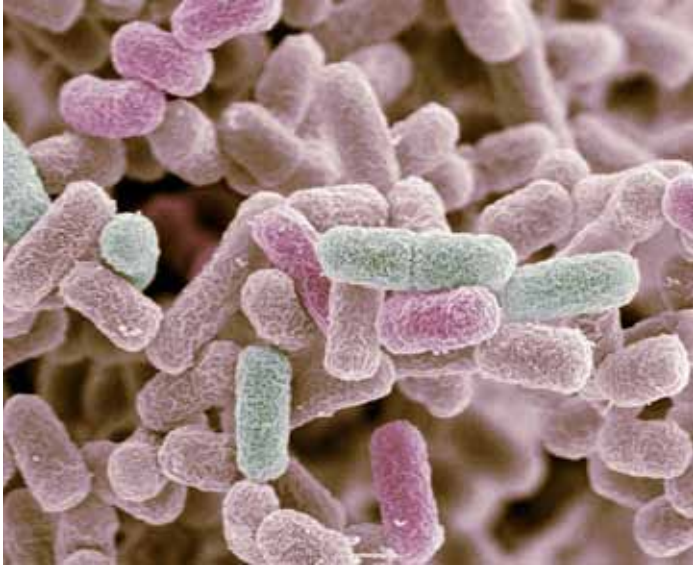
Keasling and his team modified the common

bacterium *Escherichia coli*, bypassing its existing metabolic pathways and engineering a new pathway so that it produced an important chemical precursor to artemisinin, called artemisinic acid. Keasling's team had succeeded in turning *E.coli* into a microbial factory for a new molecule it would not produce in nature. This was impressive enough in itself: but they also knew this core technology could be used to produce thousands of other molecules - including biofuels.

Instead of attempting to improve on existing biofuels like ethanol via the algae biofuel route, Keasling and his team decided to design entirely new fuels. They called these 'perfect fuels' because they would be an improvement on nature: for example, by providing more energy per litre whilst emitting fewer pollutants.

They began with a list of candidate molecules, and then narrowed them down to those that could be both developed in the laboratory and used in today's engines without modification. Although the company admits it's a long way from





producing a practical biofuel, Amyris has identified a list of compounds, including replacements for both jet fuel and diesel.

The technology is similar to Keasling's earlier work, but instead of adding genes and changing metabolic pathways so that *E. Coli* produces artemisinin acid, Keasling's team are working to change *E.coli* so it becomes a production plant for 'perfect fuel' molecules. It may sound like science fiction, but venture capitalists don't believe so: Keasling's search for the perfect fuel molecule has already raised \$20 millions in financing.

#### **Production reality**

Most researchers are candid enough to admit that there is a long way to go before microbe-produced biofuels are on sale at every garage. For example, there are problems in scaling-up from algae in laboratory flasks to industrial-sized tanks: the organisms can crowd one another out and produce toxic waste that halts the process. And, since no-one has yet produced microbe biofuels on an industrial scale, they don't know how much it will cost.

George Church concedes that developing renewable petrol on an industrial scale is a difficult proposition but, he says, "If this works out, much of the current motivation for switching away from hydrocarbons might vanish."

But the motive is there, and the researchers are finding more and more investment:

"It's not backyard inventors at this point," says George Douglas, a spokesman for the US Department of Energy. "It's people with experience to move it forward."

Other researchers are convinced that microbe biofuels will be here, soon. Solazyme, who have a development and testing agreement with oil giant Chevron, believe they can produce biodiesel on a commercial scale within a few years: "It's entirely possible that your next car could run on biodiesel produced by algae," says Jonathan Wolfson.

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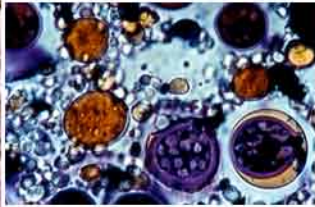
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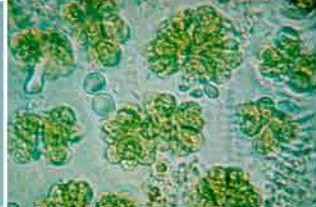
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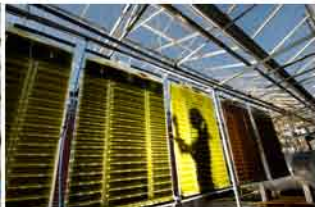
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