NEWS

Algae's Second Try

Fifteen years ago, the United States gave up on algal-based biofuels. Now synthetic biology has helped revitalize the field

IN SCIENCE, AS IN BASEBALL AND COMEDY, timing can be everything. John Sheehan learned that the hard way when he strove to make biofuels from algae from the late 1970s through the mid-1990s. The effort at the National Renewable Energy Laboratory (NREL) in Golden, Colorado, surveyed more than 3000 algal strains for their ability to produce oils that could be converted into diesel and other transportation fuels, then looked for ways to boost oil production in the best ones.

It wasn't enough. Faced with a tight budget, the U.S. Department of Energy (DOE) killed the program in 1996, opting instead to focus its limited funds on turning agricultural wastes and other "cellulosic" material into ethanol.

Fifteen years later, the algae biofuels business is thriving. Since 2000, more than $2 billion in private funds have flooded into the field. In May, Solazyme, an algae biofuels company in South San Francisco, California, raised $227 million on the stock market. Last year, ExxonMobil announced it would invest up to $600 million in the field, with up to half going to Synthetic Genomics, a San Diego, California, startup looking, like Solazyme, to use synthetic biology to create commercial fuelmaking algal strains. And DOE and other U.S. federal agencies have jumped back on board contributing hundreds of millions of dollars more, including $104 million from the recent economic stimulus package to Sapphire Energy in San Diego to build a large-scale algae fuel demonstration facility in New Mexico. Even Sheehan is back in the biofuels game again, studying the environmental impact of biobased fuels at his new home at the University of Minnesota, Twin Cities.

So why the change? In short, better timing, Sheehan says. Biotechnology has made massive strides in recent decades, now making it relatively easy to tinker with algae in ways not possible during the first flurry of interest. "The tools we had to use to manipulate algae were medieval compared to what we have today. Synthetic biology didn't exist in 1996," Sheehan says. As a result, despite algae's advantages, he and others could not overcome the high costs of obtaining oil from these organisms.

But now, companies are using synthetic biology techniques, along with other biotech and engineering advances, to bring those costs down, making algae more efficient by changing the way the organisms use light, increasing the oil content of cells, and improving their efficiency at producing fuel precursors. A spate of recent advances "gives me more assurance that this isn't just folly," Sheehan adds.

It's easy to see why plenty of scientists and investors agree with Sheehan. For starters, with fossil fuels becoming increasingly scarce, expensive, and a source of political instability, the potential market for replacing these liquid transportation fuels is worth trillions of dollars per year. Corn- and sugar-based ethanol production already has a share of that. But because a liter of ethanol has only two-thirds of the energy content as the same volume of gasoline, the alcohol isn't well suited for fueling aircraft and heavy trucks, big chunks of the transportation industry. Plants that produce more energy-rich oils for biodiesel, such as soybean and oil palm, are a better fit in those areas. But these plants produce at most 5930 liters of fuel per hectare per year, according to DOE's 2010 National Algal Biofuels Technology Roadmap.

Fast-growing algae, on the other hand, can produce between 9353 and 60,787 liters per hectare per year of fuel. And some algae companies are convinced that they will ultimately do much better than that. If such a promise comes to pass, algae farms on the scale of Colorado could produce all the gasoline used in the United States each year, a small fraction of the land that would be required for making a comparable amount of biofuels from corn or cellulose (see figure).

Furthermore, unlike most plants, some strains of algae thrive in brackish water, saltwater, or even waste treatment water. Algae farms can also be sited on land unsuited for traditional agriculture. So, in theory, large-scale algal fuel production would not interfere with food production the way other biofuels can. "The fundamental biology makes algae a massive opportunity for humanity," says B. Greg Mitchell, an algae researcher at the University of California, San Diego (UCSD).

But as Sheehan and his NREL colleagues learned early on, algae-based fuels present many challenges, ones that still make them prohibitively expensive. Despite the fact that algae grow quickly, they typically make up only 0.1% of the volume of the water in which they grow. That means collecting a kilogram of algae requires processing 1000 kilograms or more of

New crude? Synthetic biology helps researchers make high-oil-producing algal strains.

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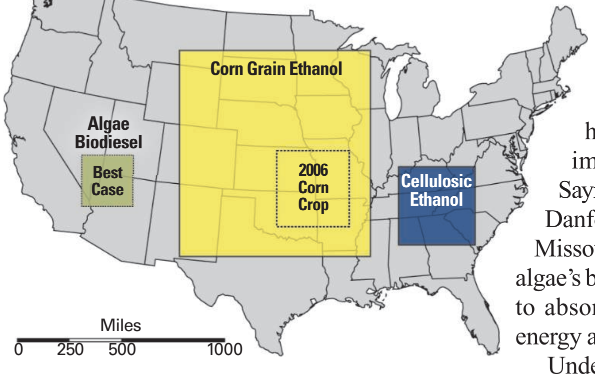
Land Required to Displace All Gasoline in the United States



Competitive advantage. Fast-growing algae yield more fuel per hectare than other biofuel producers.

water, an energy-intensive operation. The algae must be harvested and their oil extracted, collected, and processed into the final fuel. Those challenges currently make the cheapest algal fuels cost about $2.25 per liter, more than double today's average gasoline price in the United States, NREL researchers say.

Green design

How individual companies are trying to cut costs depends in part on the type of alga they use and how it's grown. The most popular strategy to grow algae is in sunny, open-air, shallow ponds. But because the algae shade each other, they grow at a low concentration and thus a large amount of energy must be spent concentrating them and harvesting the oils they produce. Competition from other algae strains that blow in and bacterial and viral infections further compromise the efficiency of open ponds.

A separate approach grows the organisms in closed chambers called bioreactors. These chambers can be either transparent, to allow the algae to grow using sunlight, or opaque, in which case the algae are fed sugars or other nutrients to promote their growth. Although bioreactors can grow algae at a greater density, they cost much more than open ponds to set up and operate.

Synthetic biology is helping researchers produce fuels more efficiently in both settings. "We can very quickly do lots of genetic manipulations with synthetic biology," says Alex Aravanis, chief technology officer of Sapphire Energy. "We can go orders of magnitude faster to discover key genes related to yield." A few years ago, the yield had been stuck, with no more than 40% of the alga's weight being oil. Now, with synthetic biology's ability to alter algal metabolic pathways en masse, rather than one gene at a time, "we have the opportunity to drive those efficiencies to unprecedented levels," says Stephen Mayfield, an algae biologist at UCSD.

Some synthetic biology efforts have gone into making indirect improvements in oil yield. Richard Sayre, a molecular biologist at the Donald Danforth Plant Science Center in St. Louis, Missouri, and his colleagues are improving algae's biofuels potential by engineering them to absorb less light, thereby leaving more energy available to nearby algae.

Under natural conditions, individual algae hog the light in an effort to outcompete their neighbors: Their light-absorbing molecular complexes, called antennas, really use just one-quarter of the photon energy they absorb. So Sayre and his colleagues inserted a new set of metabolic instructions to make the algae more community-oriented. They directed the single-celled plants to adjust the size of the antennas such that in bright light, they absorb only enough photons to make as much oil as possible, leaving the rest for neighboring cells. Preliminary studies, presented in August at the American Society of Plant Biologists (ASPB) conference in Minneapolis, Minnesota, show that the strategy increased the population's growth rate by 30%.

That's not the only algae makeover under way. Sayre's team is also boosting metabolism in algae by giving them a human gene for an enzyme called carbonic anhydrase (CA) II, which helps regulate carbon dioxide in red blood cells. In the algae, it converts an inorganic form of cellular carbon into carbon dioxide, which can then be used in photosynthesis to ultimately make oils. The human CA is far more efficient than the algae's own CA. With it, algal photosynthesis rates jumped between 30% and 136% depending on the test conditions, the team also reported at the ASPB meeting.

Gushing with oil

Numerous companies are pushing the limits of algal biology in other, poorly disclosed ways. Harrison Dillon, president and chief technology officer of Solazyme, will only say that his company has engineered algal strains with an oil content of over 80% of their weight. Those strains are grown in bioreactors and fed sugars. Last year, Solazyme produced 416,395 liters of oil and announced that it can produce algae-based fuel for less than $120 a barrel, only slightly more than the recent cost of petroleum.

Sapphire's Aravanis adds that his company is using synthetic biology to come up with and rapidly survey thousands of different genetic manipulations of photosynthetic algae in an effort to make high-oil producers. As part of that program, it has identified several genes, some from algae and some from other organisms, that when inserted into lipid biosynthesis pathways increase oil production enough that if scaled up, the algae could produce an additional 4675 to 9383 liters per hectare per year. These results have yet to be realized in field trials, but the company is currently building a demonstration facility in New Mexico that is expected to produce between 5000 and 10,000 barrels of oil per day by 2018.

Focusing on a different organism, researchers at Joule Unlimited in Cambridge, Massachusetts, meanwhile, are improving the efficiency of photosynthetic bacteria for producing hydrocarbons to make diesel. These bacteria secrete hydrocarbons, so Joule researchers are modifying the bacteria to grow more slowly and instead to divert carbon dioxide almost exclusively into making hydrocarbon fuels. According to Dan Robertson, Joule's head of biosciences, the company now has strains that routinely convert 90% of the carbon atoms that come in as carbon dioxide into fuel molecules secreted by the organisms. The company is currently operating a pilot plant in Leander, Texas, and plans to open a large-scale demonstration plant in New Mexico next year.

Test bed. Open-air "raceway" ponds grow algae cheaply but must contend with infections and predators.

With these and other innovations now taking hold, Sayre is hopeful: "I'm convinced this time around we're much smarter and have a better shot at succeeding."

–ROBERT F. SERVICE

MAP: ADAPTED FROM G. CHARLES DISMUKES *ET AL.*, *CURRENT OPINION IN BIOTECHNOLOGY*, **19** (2008); PHOTO: COURTESY OF SAPPHIRE ENERGY