Synthetic Biology Transforms Green Goo to Black Gold

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Solazyme Engineers Algae to Pump Oil

Finding substitutes for petroleum is one of the great challenges of the 21st century. The U.S. alone gulps about 225 billion gallons of gasoline and other oil-derived fuels annually. Known crude oil sources are tapped to the max against demand from developing, as well as developed, countries. But advances in synthetic biology are now enabling scientists to transform bacteria, algae, and fungus into fuel producers that could power tomorrow's cars and planes.

In a South San Francisco industrial park, scientists at Solazyme (http://www.solazyme.com) are concocting oil from algae. Inside the lab, flasks of bright green algae shimmy on vibration tables. Outside in the parking lot, a 2005 Jeep Liberty waits for its next fill-up of algal diesel. Solazyme's algae diesel passed the American Society for Testing and Materials (ASTM) D-975 specifications in 2008. Solazyme is now perfecting its next product—jet fuel.

In sourcing strains, Solazyme's scientists leverage algae's stupendous genetic diversity. "We like to find things that are already making what we want," said Harrison Dillon, Ph.D., Solazyme CTO. "It saves us steps and allows us to take advantage of an organism's natural tolerances. Synthetic biology isn't this huge step change; people started inserting genes into micorganisms [in] the late 1970s. But the degree of what you can do has changed."

Will Pump Oil for Food

Solazyme engineers its algae to grow in the dark in tanks on a diet of sugar. Essentially, Solazyme converts previously captured photosynthetic energy contained in sugars into algal oils. Its heterotrophic algae can produce up to 75% of their mass in oil within few days, far beyond what is possible through direct photosynthesis. In addition to sugar from sugar cane, Solazyme feeds its algae pretreated bagasse (leftover cane), corn stover, and sugarbeet pulp, as well as waste glycerol from biodiesel production. Cellulosic feedstocks undergo the same pretreatment process used to generate monosaccharide sugars for ethanol.

The algae grows faster in tanks than in ponds, as it is not limited by shading from surface layers. It also can be grown at greater density, requiring less water and simplifying separation. The tank environment can be controlled for invasive organisms, pH, and salinity and water can be recycled.

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Growing algae in tanks somewhat bypasses the concern that synthetic organisms grown in ponds could exchange genetic material with local species. The potential of plug and play biology is enormous, but critics fear genetically engineered organisms could cause havoc as bioweapons or become invasive species.

According to Dillon, a gallon of oil produced by tank-grown heterotrophic algae can cost 1000 times less than oil produced by pond-grown algae, and is comparable to ethanol from sugarcane at $1.50 per gallon (1 gallon equals 3.78 liters). He estimates that the company can manufacture algal oil at costs competitive with $50–$80 per barrel (42 gallons per barrel) of oil within 24–36 months and notes that algal fuel is close to carbon neutral.

Dillon and Jonathan Wolfson cofounded Solazyme as a biofuels company in 2003. Dillon's interest had been sparked by the Aquatic Species Program at the National Renewable Energy Laboratory (NREL). Sand Hill Road venture capitalists were initially slow to appreciate biofuels, but in 2007, the company closed a Series C investment round of $45.4 million. Solazyme entered into an agreement with Chevron Technology Ventures to engineer biofuel feedstocks in January 2008.

Solazyme initially investigated algae to produce hydrogen but abandoned the idea as incompatible with existing infrastructure. The company is now leasing large-scale fermentation facilities and pipelines. Solazyme is also pursuing other applications of algae technology such as high-value plastics, edible oils, and cosmetic products.

Renewed Interest in Renewables

The National Renewable Energy Laboratory's (NREL) Aquatic Species Program is being resurrected now and the Defense Advanced Research Projects Agency (DARPA) is funding algal jet fuel. At the end of 2008, the Department of Energy (DOE) and the United States Department of Agriculture (USDA) announced the intention of investing up to $25 million over four years for biofuels research and development, and more stimulus funds are on the way.

"You can now synthesize entire genes and even pathways rather inexpensively and put them in an organism to give it functionality that it never had before," said Al Darzins, senior group manager at NREL. "But before applying such technology tools successfully, you need to know more about algal lipid synthesis pathways and how those pathways are regulated." Aquatic Species Program researchers genetically modified algae during the early 1990s before the program was shut down. However, their efforts didn't achieve greater oil production because they didn't understand how the algal lipid trigger worked.

According to Darzins, a company like Solazyme will ultimately have to feed its algae cellulosic biomass. "What techno-economical analysis shows is that it all comes down to process costs," said

Darzins. ''Right now we are estimating that if you grow algae in an open pond, your costs could be as high as $10 to $20 to produce a gallon of algal oil. For a closed system, the cost is estimated at $20 to $40 a gallon, based on only a very limited number of projected technologies.''

Darzins thinks genetically modified algae could conceivably be grown in open ponds as long as a type of biological control mechanism is engineered in, such as only growing within a very specific range of salinity.

Building algal production facilities to the necessary scale would be a huge undertaking. Darzins estimates that to get three billion gallons of soybean oil (soybeans can produce 50 gallons of oil per acre per year) would require about 62.5 million acres of land. Algae producing 1200 gallons per pond-acre per year would only need 2.5 million acres of land. ''While this sounds like a lot, 3 billion gallons of algal oil would only displace about 5% of our diesel needs.'' said Darzins.

Following Different Pathways

Critics say that biofuels can also exact an environmental price as forests are turned into farmland to grow feedstocks. It is debatable just how energy-positive certain biofuels are, but hope is being pinned on deriving better feedstocks and fuels from plant cellulose at places like the Joint BioEnergy Institute (JBEI; http://www.jbei.org) in Emeryville, CA, where researchers are deconstructing biomass, as well as engineering plants to be more degradable, and microbes to transform sugars into fuels compatible with current transportation infrastructure. ''There is a significant need for synthetic biology in all these areas, particularly in fuel synthesis.'' said Jay Keasling, CEO and vice president of Fuels Synthesis at JBEI.

According to Keasling, while the common functional components of biological systems, commonly referred to as ''parts'' by synthetic biologists, are interchangeable, they are not necessarily standardized, nor are the connections between them fully characterized. Synthetic biologists attempt to characterize the behavior of the parts as well as set standards for characterization of connections. ''An additional problem is composability,'' Keasling said. ''Once you put components together and they don't exactly behave the way you want them to, you can use evolution if you've got some sort of screen or selection to get them to work together better.''

''We are at a stage in synthetic biology that we shouldn't accept what life has given us,'' Keasling said. ''We should engineer the cells that would produce the kinds of fuels that fit into the $3 trillion transportation infrastructure we have in this country.'' Amyris (http://www.amyris.com), a company Keasling founded, started with a malaria drug (*Wolfson, 2005*) but now is engineering *E. coli* to produce hydrocarbon molecules using the isoprenoid pathway, as well as biodiesel and jet fuel.

Advanced biofuels companies can either reorganize genes to make an existing organism more efficient or build one from scratch. However, there are many genes that have unknown functions. Keasling praised Craig Venter's company Synthetic Genomics (http://www.syntheticgenomics.com) for constructing organisms de novo, but he says that most companies attempt to take the shortest path to a working organism by starting from a living system that already exists.

LS9 Teaches *E. coli* New Tricks

South San Francisco-based LS9 (http://www.ls9.com) engineers *E. coli* and other organisms to convert sugars to fatty acid-derived compounds such as alkanes and esters for fuels, as well as higher value commodity chemicals such as fatty alcohols and olefins, rather than building bugs from scratch. ''We have focused the majority of our effort on fatty acid metabolism, since it is one of biology's most energetically efficient pathways and is used for both the production of cellular structural components such as membranes and energy storage compounds such as fat,'' said Stephen del Cardayre, vice president, research and development. ''It is also amenable to engineering, allowing diversion from the normal pathways to structural lipids to modified compounds that can be used as fuels and chemicals.'' The venture-funded company was started three years ago by George Church, professor of genetics at Harvard University; Chris Somerville, director of the Energy Biosciences Institute and professor of plant and microbial biology at the University of California, Berkeley; and Jay Keasling.

LS9 currently feeds its microbes sugar from sugarcane, burning the bagasse for energy as an interim step to using cellulosic technology. In what del Cardayre dubs ''microrefineries,'' LS9 conducts all chemical conversions in a single cell in one step. ''We use synthetic biology to identify genes that encode key enzyme catalysts,'' said del Cardayre. ''Those genes that express these catalysts are compiled inside our cells.'' Cells produce a finished fuel product that is centrifuged and needs no additional transformation.

LS9 has scaled from a lab output of 2 to 5 liters to a 1000 liter pilot plant. The company plans ramping up production this summer in a commercial 100,000 liter plant. While the companies profiled here currently use sugarcane as a feedstock, it isn't cheap enough long term. ''For this entire industry to have the impact that it needs to have, we all have to be working with cellulosic feedstocks,'' said del Cardayre. Cellulosic conversion technologies, however, are not yet commercially ready.

''Our goal is to produce fuel, diesel initially, at a cost competitive with $45 barrel oil,'' said del Cardayre. ''This is without a subsidy and is based on the current price of sugar cane.'' LS9's projections are based on 100 million gallon per year production plants.

While the renewable fuels field is currently flush with hype and investment, the real costs and yields are still unknown until companies embark on large-scale production. Significant process issues like separation and scale-up remain. But the proof is in the pudding or, in this case, in the vat.

REFERENCE

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