Biotech's green gold?

Algae have long been touted as a rich and ubiquitous source of renewable fuel but thus far have failed to be economically competitive with other sources of energy. Could new advances change that? Emily Waltz investigates.

When the UK's Carbon Trust last year set out to fund algal biofuels research, organizers quickly met with a mélange of overzealous claims coming from the industry. Companies were projecting biofuel yields ten times what is theoretically possible and proposing techniques that are not now and may never be economical. A year later, after wading through the claims and gathering opinions from a network of more than 300 experts, the agency announced on October 23 the creation of the Algae Biofuel Challenge, a £16 ($24) million fund that will support the development and large-scale production of algal oil.

The Carbon Trust's experience navigating algae excitement is one that funding groups and investors in the biofuels industry increasingly face. Encouraged by high oil prices and the push for alternative fuels and carbon trading, more than 100 such algae-to-fuel companies have popped up worldwide, mostly in the last couple of years, say industry experts. But, not a single commercial facility has been built, and an eagerness to be the first, plus the enticing investment along the way, has encouraged some entrepreneurs to overstate their capabilities. "Some of these operators and startup companies claim they can do anything under the sun," says Tasios Melis, an algae researcher at the University of California, Berkeley.

Among startups in any nascent technology, there will be some winners and a lot of losers. But when fuel is the product, particularly apt is the old admonishment: buyer beware.

Feeding frenzy

Nearly every week the industry sees another anecdotal example of algae fervor. Investors in August plunked more than $45 million into S. San Francisco–based Solazyme, an algal fuel company using 'synthetic biology'. The launch of a new startup, AXI, spun out of the University of Washington in Seattle, was also announced in August. In September, Sapphire Energy, a San Diego startup developing crude-like oil from algae, said it had raised more than $100 million. At its annual biotech meeting in October in Hanover, Germany, European Bioperspectives for the first time held algae sessions.

The draw is that algae have the potential to produce up to ten times more oil per acre than traditional biofuel crops such as oil palm (**Table 1**). They can survive where agricultural crops can't, such as in salt water and on marginal land. They thrive on a diet of waste carbon dioxide and the nutrients in agricultural run-off and municipal wastewater. They aren't needed for human food. And in addition to fuels, valuable co-products, such as biopolymers, proteins and animal feed can be made during the process.

The concept of using algae to make fuel was first discussed more than 50 years ago but a concerted effort began with the oil crisis in the 1970s (Hu, Q. *et al. Plant J.* **54**, 621–639, 2008). The US Department of Energy (DOE) from 1978 to 1996 devoted $25 million to algal fuels research in its aquatic species program at the National Renewable Energy Lab (NREL) in Golden, Colorado. The program yielded important advances that set the stage for algal biofuel research today.

In the 1980s and 1990s, researchers tried various approaches. They grew algae in outdoor open ponds and enclosed photobioreactor tanks, they experimented with breeding, they fed algae smokestack carbon dioxide emissions to boost their growth and tested species that can tolerate extreme salt and pH environments. The first genetic transformation of microalgae came in 1994, and scientists a few years later successfully isolated and characterized the first algal genes that express enzymes thought to enhance oil production. From 1990 to 2000, the Japanese government funded algae research through an initiative at the Research Institute of Innovative Technology for the Earth (Kyoto). The program focused on carbon dioxide fixation and improving algal growth with concentrated mirrors that collect light.

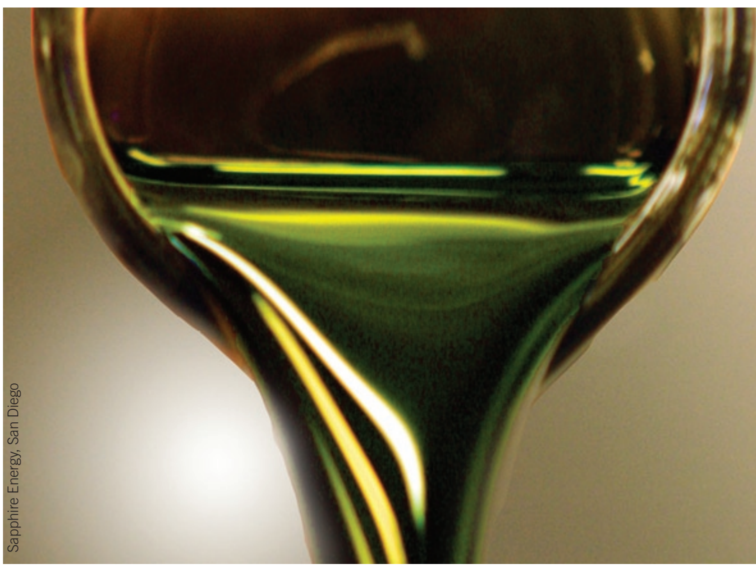
These approaches yielded some successes and many are still the focus of scientists today, but none have proven economical on a large scale. The DOE program closed in 1996, in part, because algal systems couldn't compete with the cheap crude oil of the late 1990s. Now, NREL is back in the algae business with a cooperative research agreement with Chevron, of San Ramon, California, and the beginnings of a research funding roadmap it is designing for the DOE. Oil prices, which reached all-time highs last summer, renewed many scientists' hopes that the economics might work.

Only a handful of companies are working with genetically modified algae but among the small group are some of the most serious and promising in the algae-to-fuels industry, say independent researchers (**Table 2**).

Pond scum 101

Like all photosynthetic organisms, with a little water, a few nutrients and carbon dioxide, microalgae—pond scum—use energy from the sun to grow. With just these inputs, they can easily double their population in a day.

Faced with stresses such as nutrient deprivation, algae put their energy into storage—often in the form of natural oils such as neutral lipids or triglycerides—and growth slows. Similar to the oils from crops such as soybeans, jatropha and oil palm, algal



As food crops become unpalatable as a source of biofuel, can algae take their place?

oil can be extracted from the organisms and refined into biodiesel (methyl (ethyl) esters) by transesterification with short-chain alcohols (e.g., methanol) or by esterification of fatty acids. Algae also synthesize other fuel products, such as hydrogen, ethanol and long-chain hydrocarbons that resemble a crude-like oil.

But when algae divert energy into accumulating oil, they don't grow very fast, if at all, and when they devote energy to growing, they don't make much oil—a trade-off that can result in little increase in overall production of oil. "It's simply a law of thermodynamics. You can't get around that," says Steve Mayfield, a biologist at The Scripps Research Institute in La Jolla, California.

This trade-off has stumped scientists for decades. Companies are still trying to improve on concepts and cultivation systems developed in the 1980s. "I worked on biofuels 20 years ago and nobody gave a damn," says Keith Cooksey, a semi-retired microbiologist at Montana State University in Bozeman. "Then about a year ago I started getting phone calls and requests for papers I had published in the 1980s. That doesn't usually happen. Either you've contradicted what you published 20 years ago or the field has moved on and it's irrelevant. But these companies and programs are basically picking up where I left off."

Since the mid-1990s, however, the tools for genetic engineering have improved, and scientists are increasingly applying them to algae with fuel applications in mind. Much of the work is focused on identifying the genes involved in lipid synthesis and how those genes are regulated. The idea is to manipulate those genes so that the organisms' metabolic pathways are tricked into producing storage lipids, even when the algae are not under stressful conditions. "There are none I can say is a 'key gene' but there are a lot of labs looking for it," says Mayfield, who is studying *Chlamydomonas reinhardtii*.

C. reinhardtii is the *Escherichia coli* of algae and the model organism for many labs. It is one of only a handful of microalgal species whose nuclear and chloroplast genomes have been sequenced. But 'Chlamy' isn't the ideal biofuel organism because it doesn't naturally produce much oil. "The choice to sequence algae is not being made because [of their] importance in biofuels applications," says Al Darzins, a group manager at NREL. "But I think the [Walnut Creek, California-based] Joint Genome Institute is becoming more interested in that."

Algae species are spectacularly diverse, which makes for slow genetics research because the knowledge often doesn't translate from one species to another. But this diversity also gives scientists hope. Up to 200,000 algal species may exist, Darzins says, and less than a quarter have been described. The idea that there are great species still out there keeps scientists hunting for them, but it's a laborious process.

Table 1 Potential oil yields per acre per year

Crop	Gallons of oil/acre/year
Soybeans	43
Sunflower	86
Canola	171
Jatrjopha	214
Palm oil	641
Microalgae	up to 6,000 (with future technology)

For example, the US Air Force funded Juergen Polle, a biologist at Brooklyn College in New York, to do algae bioprospecting. Polle and his students go on local excursions and trips to the western US to collect species. They bring them back to their lab, and one at a time, they isolate strains and screen them for lipids. Stacks of Petri dishes fill the back room of Polle's lab. High-throughput methodologies, such as cell sorters and robotic liquid handling, promise to speed up the process.

> "People are extrapolating inappropriately from lab data," says Robert Trezona, head of R&D at the Carbon Trust in London.

Polle and others are looking for strains that not only produce oil, but also fend off other organisms. Algae cultivated in open ponds are subject to predators and other, stronger algal species. Transgenic algae are particularly at risk because their commercially important traits may reduce their fitness. As there are likely fewer algal predators in extreme conditions, some researchers theorize that transgenic extremophiles may have a better chance of survival than other types of transgenic algae.

Enclosed photobioreactors provide a bit more protection for transgenic algae, although these systems are no fortress. Closed bioreactors tend to be more productive than open systems, but are more expensive. Selling the co-products made during the process will be necessary if any of the technologies are to be economically viable.

The numbers

Near-term technologies may allow algae to produce up to 6,000 gallons of oil per acre per year (gal/ac/yr). "If you really push the limits, then maybe 10,000 gallons per acre," says Ron Pate, a researcher at Sandia National Laboratories in Albuquerque, New Mexico. This figure could improve with advances in cultivation, species selection, breeding and genetic modification, but only to a certain extent. The laws of thermodynamics and the limits of photosynthetic efficiencies just won't allow it. "When you see 20,000 or beyond—that's total bologna," says Pate. "It isn't going to happen."

Yet there are companies claiming they can make up to 100,000 gal/ac/yr, and raking in tens of millions in investment based on those promises. "The moment their production goes over a certain prediction of gallons per acre, you know they are not serious," says Polle, who has supplied algal strains to startups. "Only a handful of companies are really serious."

Valcent Products, a public company located in Vancouver, is experimenting with a range of algal species in enclosed bioreactors. Valcent CEO Glen Kertz says he can sell a barrel of algal oil for less than a barrel of crude oil and that his system has the potential to produce 100,000 gal/ac/yr.

"I said to him [Kertz], 'You are not doing anyone any favors by making absurd claims'," says Scripps' Mayfield. "That is five times the theoretical maximum energy from sunlight landing on an acre. It's physically impossible to do that." No outside experts have been allowed to validate the system yet, according to Kertz.

In Valcent's 200-square-foot test facility in El Paso, Texas, algae flow through 30 clear plastic panels hung vertically inside a greenhouse. The algae are exposed to light for a few minutes and then pumped into dark, underground tanks. Kertz theorizes that algae can absorb more light if they are repeatedly exposed to it for short periods, rather than if they are left in the sun all day.

"Ridiculous nonsense," says John Benemann, an algae-to-fuels consultant and a former researcher at the University of California, Berkeley. Benemann says that the technique may have some effect on productivity, but not enough to support the company's claims.

Valcent plans to build a 100-panel demonstration system, and will then seek to license its

Table 2 Companies applying genetic and genomic techniques to harvest fuel from algae

Company	Technology	Investment; investors
Aurora Biofuels (Alameda, California)	Overexpressing genes, such as carboxylic acetylcoenzyme A, to improve triglyceride synthesis; may focus on strains native to facility's location.	$25 million; Gabriel Venture Partners (Redwood Shores, California), Noventi (Menlo Park, California), Oak Investment Partners (Westport, Connecticut)
Algenol	Metabolically enhancing cyanobacteria to directly synthesize ethanol; expressing enzymes pyruvate decarboxylase and alcohol dehydrogenase II.	$70 million; firm's founders; license with Biofields (Lomas de Chapultepec, Mexico) for undisclosed fee and royalties
Sapphire Energy	Producing an undisclosed 'crude-like oil' that can be refined into gasoline or jet fuel. Experts say some of their organisms are genetically engineered, but the company has not yet publicly confirmed this.	$100 million in 2008; ARCH Venture Partners (Chicago), The Wellcome Trust (London), Cascades Investments (Kirkland, Washington; Bill Gates' personal investment vehicle)
Solarvest BioEnergy (formerly GCH Capital Partners, Vancouver)	Hydrogen production in a single cycle from algae; plastid engineering through introduction of promoters that target proteins involved in photosynthesis (e.g., psbA and psbD), hydrogenases (e.g., HydAl and HydA2) and proteins involved in their regulation (e.g., HydEF and HydG)), developed at the University of Geneva (WO Patent 2008/021223 A2) and PhycoBiologics of Indianapolis, Indiana.	Publicly traded on the TSX Venture Exchange
Solazyme	Use of antisense and RNA interference to regulate light-harvesting genes (e.g., Lhca2 and Lhcbm4), chlorophyll biosynthesis genes (e.g., hydroxymethylbilane synthase) and signaling genes (e.g., tial), together with synthetic genes containing unnatural codons to maximize triglyceride production through fermentation (US Patent 2008/0124756 A1). Business plan is to target nutraceuticals market, then biofuels.	$70 million; Roda Group (Berkeley, California), Harris & Harris Group (New York), Lightspeed Venture Partners (Menlo Park, California), Braemar Energy Ventures (New York), Chevron Technology Ventures (Houston)
Synthetic Genomics (La Jolla, California)	Modifying genes to create new secretion pathways through the outer membranes of algae so they expel the oil, making harvesting easier. Patent describes genome assembly technology in which cassettes of algal genes involved in triglyceride productions are cloned and rebooted into appropriate recipient prokaryotic cells (WO 2008/144192 A1).	Valued at $300 million according to co-founder Craig Venter. Investors include founders, BP, Biotechonomy (Boston), Draper Fisher Jurvetson (Menlo Park, California), Desarrollo Consolidado de Negocios (Monterrey, Mexico), Gethang Berhad (Malaysia), Meteor Group (Palo Alto, California)

technology, says Kertz. The company does not plan to build full-scale facilities or sell oil.

Such small-scale facilities are typical among algal biofuels companies, which may be a driver behind some inflated claims. "People are extrapolating inappropriately from lab data," says Robert Trezona, head of R&D at the Carbon Trust in London.

Making outrageous claims has gotten algae companies into trouble in the past. Two years ago, De Beers Fuel in South Africa (unrelated to the diamond company) said it would within five years produce more than 6 billion gallons of algal biodiesel per year. The company collected tens of millions of dollars from investors, but the biodiesel facilities never materialized. A group of investors, a marketing company and a security company reportedly filed a claim in a South African court, accusing company executives of misappropriating funds.

De Beers Fuel based its claims, in part, on an obsolete bioreactor it had bought from Cambridge, Massachusetts-based GreenFuel Technologies. "We were not careful about our prospective buyers," says Bob Metcalf, a partner at Polaris Venture Partners in Waltham and an investor in GreenFuel. "The South African company said they wanted to buy it for $300,000 and use it for demonstration. We stupidly agreed."

GreenFuel designs closed bioreactors that feed off industrial flue emissions and, in 2007, faced some setbacks. A pilot project failed, its bioreactors turned out to be twice as expensive as expected and the company had to fire nearly half its staff, according to Metcalf. For the failed pilot, the company had made the mistake of trying to scale-up a small test system by a factor of 100 all in one step, and it didn't work, he says. Some experts have also expressed skepticism about the oil yields GreenFuel has projected in the past, which have since been lowered, according to people familiar with the matter. GreenFuel spokespersons could not confirm the old projections.

> "The old algae world has produced some old-timers who are negative. We're trying not to listen to them," Bob Metcalf, Polaris Venture Partners.

GreenFuel faces the same challenges that most closed system operators face. Photobioreactors are more complex and more expensive than open ponds, and it's tough to make the economics work (**Box 1**). "Anyone working on closed photobioreactors has got a problem," says Benemann. "And there are dozens of these companies out there," he says. "Just like in agriculture, you have to keep it as simple as possible and as cheap as possible. You can't grow commodities in greenhouses and you can't grow algae in bioreactors."

An informal calculation by Pate and his colleagues at Sandia estimates that closed systems double the cost of a gallon of algal oil, says Pate, noting that their calculations were based on limited data. For example, oil from an open system may cost $10–20 per gallon to make and $20–40 per gallon in a closed system, he says.

"I don't think we'll ever get down to $3–4 per gallon," adds Mayfield, who chairs the scientific advisory board at Sapphire Energy. "Realistically, we could get it down to $6–10 per gallon in the next four to five years."

Startups pooh-pooh the naysayers, of course. "The old algae world stemming from the failed project at NREL has produced some old-timers who are negative," says Metcalf. "We're trying not to listen to them."

Most algae-to-fuel companies refuse to reveal much information about their technologies, which has led to more skepticism. "There are a lot of companies' claims that

Box 1 The economics of closed bioreactors: a back-of-the-envelope calculation

Algenol Biofuels in Naples, Florida, has metabolically enhanced cyanobacteria, or blue-green algae, to directly synthesize ethanol—one of the few, if only, companies working with direct production of this fuel.

The company has designed enclosed photobioreactors and in June announced it had licensed its technology to Biofields of Lomas de Chapultepec, Mexico. Biofields says it has committed $850 million to building the facility on 102,000 acres in the Sonoran Desert, where by 2012 it intends to produce a whopping one billion gallons of ethanol per year.

Algenol estimates that its technology can produce 10,000–12,000 gal/ac/yr of ethanol in the near term. But a simple calculation makes it hard to believe those yields will make Algenol's bioreactors economical. The company's current bioreactor design looks like a soda bottle turned on its side, and is about 3 feet wide and 50 feet long, says Paul Woods, CEO. At that size, the company could at most fit 290 bioreactors on an acre. At 12,000 gal/ac/yr, each bioreactor would produce 41 gallons per year. If the company could sell that ethanol for $5 a gallon—more than the going rate—it would make $205 per bioreactor per year.

Surely each of those bioreactors costs more than $205 per year to operate, especially if the cost of designing and building them is included? Woods' answer: no. He says he hopes they can maintain the bioreactors for next to nothing. The company's bioreactors will require less maintenance than other designs, he says, because the algal culture is static. Rather than harvesting algae, squeezing out the oil and growing new culture, as with lipid oils, the company waits until the ethanol is released from the organisms naturally and evaporates into the head space of the bioreactors, and then collects it.

I'm having a hard time validating what they're doing because they aren't willing to show it," says Pate at Sandia. "One example of this—and I won't say who it is—I talked to them a year ago and we even entered a nondisclosure agreement but I kept getting the runaround. That makes me skeptical of their fabulous claims."

The bubble

The danger of overly optimistic claims is that they pressure the rest of the industry to match and create expectations among investors and the public. If those expectations aren't met, the whole field could suffer—from basic research to commercial endeavors—and bullish venture capital investments fuel those expectations.

"My worries are that VCs [venture capitalists] are not considering all of the challenges and barriers that need to be overcome before this technology can be commercialized," says Darzins. "It would be horrible if a lot them jumped in with a lot of money, and then one or two years down the road realized it's harder than they thought and dropped support. That would be a tragedy."

"We should be careful with what we're promising," adds Otto Pulz, a researcher focused on photobioreactors at IGV Institut für Getreideverarbeitung in Bergholz-Rehbrücke, Germany. "I'm afraid this short-term thinking is damaging to microalgae research."

Investors have various strategies for choosing companies and conducting due diligence. Vinod Khosla, founder of Khosla Ventures in Menlo Park, California, says his criteria for all biofuels technologies is that they can compete—unsubsidized—with oil at $50 per barrel. That's a tough standard, and the dozen or so algae companies that Khosla has evaluated have not met the financial hurdles, he says. "It's not an economical technology yet," he says, "but it's worth pursuing."

Other investors look for tangible progress, such as pilot facilities. "We're looking for assets on the ground that the management team has invested in," says Tyler Krutzfeldt, a managing director at Mont Vista Capital in Miami, whose firm conducts due diligence on algae companies.

The Carbon Trust's approach was to establish a core group of seven to eight experts who helped identify yields that are feasible and technologies that are economical. They then built a community of about 300 people with different perspectives and held meetings, but were in "listening mode," says Trezona. After a year, they were able to draw some conclusions. Photobioreactors are best left as tools for labs and not for large-scale projects, says Trezona. "We think open ponds are the way to go," because the economics are better, he says.

Pate says he is trying to put together an assembly of researchers, companies and government representatives to create an open but intellectual property–friendly environment where people can discuss technologies and report R&D problems. The group last met in August.

If all else fails, the back-up plan for some companies is to produce specialized algal oil for nutraceuticals and cosmetics, and to sell byproducts such as ingredients for animal feed. A joint project between GreenFuel Technologies and the Spanish environmental company Aurantia Group of Madrid will set its sights on "higher value ingredients for feed and food" before attempting to produce biofuel, according to a GreenFuel investor, Metcalf. "The company was very much oriented toward fuel in the beginning but over the last couple of years we've been seeing that algae have other great applications," says GreenFuel CEO Simon Upfill-Brown. Such products can fetch high prices, but some of these markets already have dominant players. Alternative fuel boosters hope a plan B won't be necessary.

Emily Waltz, New York