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Future Oil

The Cargill salt ponds south of San Francisco.

Courtesy of Cargill Inset: Courtesy of Solazyme

Biofuels made from algae are the next big thing on the alternative energy horizon. But can they free us from our addiction to petroleum?

By Bob Grant

Near the southern horn of San Francisco Bay, hectares of shallow ponds the color of blood, pumpkin pie, and murky emerald stretch out across crusty salt flats in an aqueous patchwork. The tang of salt air swirls through the autumn air. A flock of seagulls laze on an earthen dyke separating two rectangular pools filled with the Bay's backwater. Scrubby hills stretch beyond one pond's salty banks.

The Cargill food company manages these evaporation ponds, used to produce salt for more than a century. But one day, these ponds could be important for other reasons.

The calmness of the scene is belied by vortices of colorful, microscopic algae, churning in the water.

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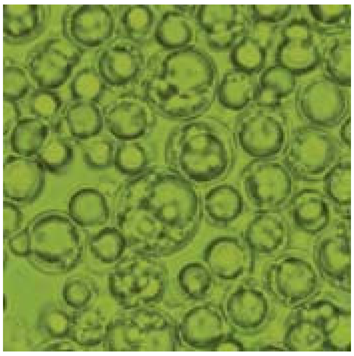
The latest crop of biofuel pioneers are looking past corn and french fry grease to microscopic organisms which they hope to coax into producing fuels to power planes, trains, and automobiles. At first, biofuel experts focused their attention on ethanol from the sugars in corn kernels; next, heads turned to second generation biofuels, such as ethanol from the cellulose in non-food plant parts. Now the next, or third, generation is here.



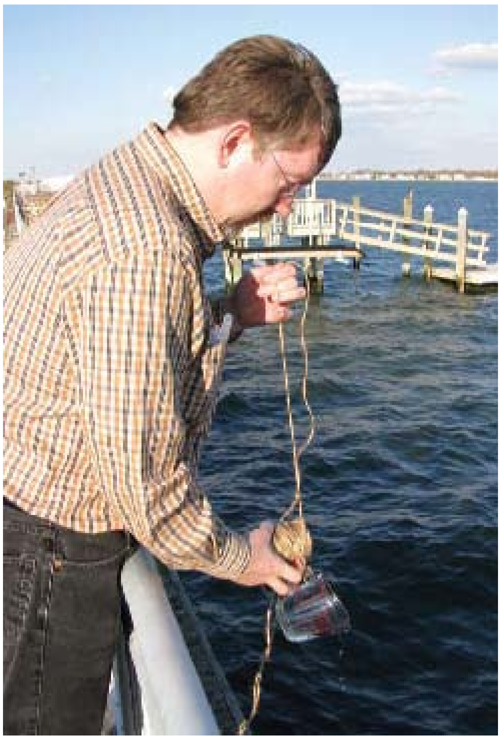
Algal cells pregnant with oil globules.

Courtesy of Solazyme

"We've really seen an explosion in third generation biofuel companies and ideas," says Matt Carr, director of the industrial and environmental section at the Biotechnology Industry Organization. "Algae is the hottest in terms of buzz."

The basic concept behind algal biofuels is deceptively simple. Microalgae naturally produce and store lipids similar to those found in most vegetable oils. If scientists can genetically jigger the oil-storing tendencies of algae into becoming more efficient than they are in nature, commercially viable levels of transportation fuels may result. The key challenges include selecting the most suitable algae strains, growing these algal cells at optimal rates, engineering the metabolic pathways that control oil production to create cells pregnant with desirable oil products, and extracting the oil in an efficient and economic manner.

It's not the first time algae have been pegged as a fuel source: Between 1978 and 1996, the US Department of Energy explored the potential of algae, but stopped when the price of a barrel of crude oil fell from $50 to $20. Not since then has there been so much research and development focused on making algal biofuels a broad reality. Dozens of private companies and a few publicly-funded researchers are now working on algae strains similar to those contained in the Cargill ponds, trying to bring the cost of algal oil manufacture down to levels that could save consumers from the roller coaster of gasoline prices. Key players in the algal fuel race include Solix Biofuels, a Colorado-based operation which plans on firing up a closed-tank bioreactor system that uses waste carbon dioxide from beer making, and Aquaflow Binomics, a New Zealand company that seeks to produce biofuels by harvesting wild algae from polluted waterways. Earlier this year, in the first algae-powered commercial aircraft test flight, a Continental Airlines Boeing 737 was powered in part by an algal biofuel produced by California-based Sapphire Energy.



Polle uses a homespun appartus to sample algae rich waters near Brooklyn.

In the fading light over South San Francisco Bay, one possible solution to our costly oil addiction splashes color across the landscape. Is this the oil field of the future?

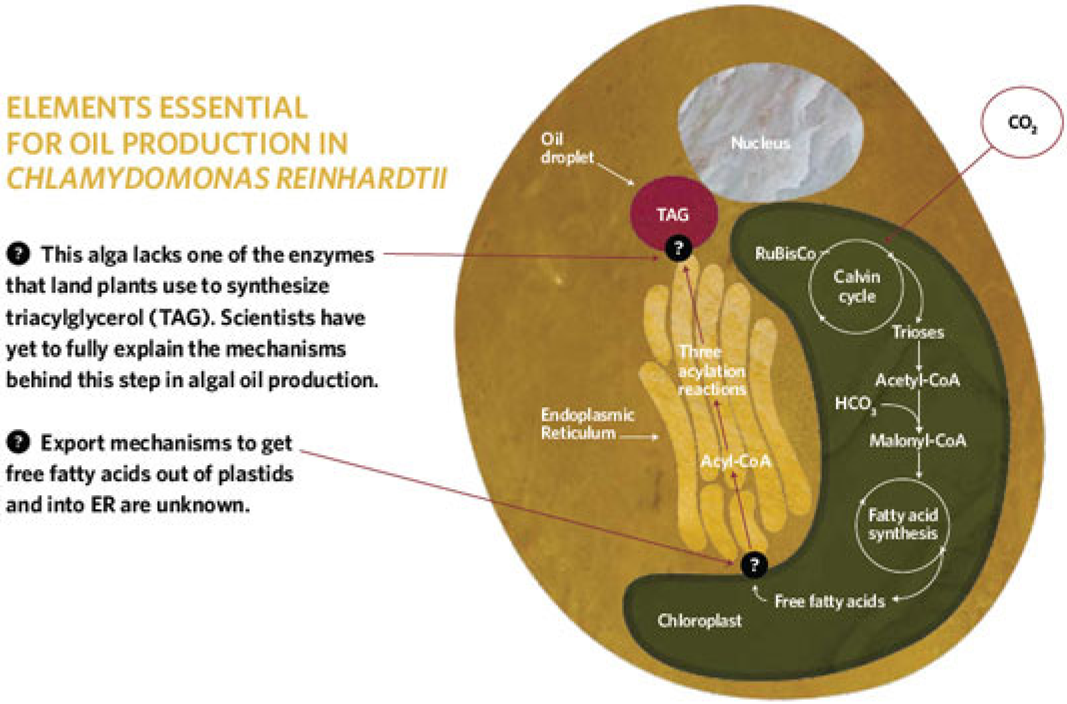
On a clear, sunny November morning, Juergen Polle dips a disposable dropper into a sloshing slough of Sheepshead Bay on Brooklyn's southern shoreline, searching for microalgae. "On average, I get three to ten strains per water sample," he says. Polle escapes his fourth-floor lab at Brooklyn College every chance he gets to sample the waters surrounding Brooklyn and Long Island, on the hunt for species that might one day serve as the tiny engines of a biofuel-based economy. (Still, he's a little reluctant to call himself an algae hunter. "If you want to put it in two words, then yes," he says.)

Down the road from his first sampling site, Polle holds a ball of twine and gingerly lowers a glass measuring cup over a flood wall into more of the murky waters that surround Brooklyn. "Why do I need all those expensive tools?" he jokes.

"Why do I need all those expensive tools?" Polle jokes.

Specifically, Polle is looking for efficient oil producers, algae that can accumulate anything more than 30% of their body weight in oils. His work, now a year and a half old, is funded by a US Air Force grant that aims to develop algal jet fuel. According to Walter Kozumbo, manager of the Air Force Office of Scientific Research's bioenergy program, the Air Force uses about 2.5 billion gallons of jet fuel per year. "Clearly there's a national defense issue here with depending on foreign oil," Kozumbo says.

Polle says that since he started collecting algae for the Air Force project, he's isolated approximately 300 strains of unicellular algae, and is in the process of parsing out a few hundred more strains that are clumped together in additional water samples.


ELEMENTS ESSENTIAL FOR OIL PRODUCTION IN *CHLAMYDOMONAS REINHARDTII*

❶ This alga lacks one of the enzymes that land plants use to synthesize triacylglycerol (TAG). Scientists have yet to fully explain the mechanisms behind this step in algal oil production.

❷ Export mechanisms to get free fatty acids out of plastids and into ER are unknown.

The beauty of imagining microalgae as tiny fuel factories is that the compounds they naturally manufacture are chemically similar to petroleum-based fuels. For example, Kozumbo says, the triacylglycerides that photosynthetic algae accumulate generally resemble JP8, the kerosene-based jet fuel of choice for military aircraft. And these unicellular plants don't just make and store these useful oils; they can really crank them out. The US DOE says that microalgae have the potential to produce 100 times more oil per acre than any terrestrial plants, including soybeans.

Polle's hunt has taken him across the country, from ponds and birdbaths in Texas to the Salton Sea of California. Polle has his sights set on collecting in the salt flats outside San Francisco as they likely harbor interesting marine species that he has not yet seen.

In similar salt flats near the Great Salt Lake in Utah, Polle found a few strains of algae that he thinks might be new to science, though the constraints of his mandate prevent him from fully exploring these potential taxonomic additions. "At this point we're not really identifying them," he says. "[Taxonomic identification] is not interesting to the Air Force. We just go out and try to find the greatest diversity there is and screen for lipid production potential."

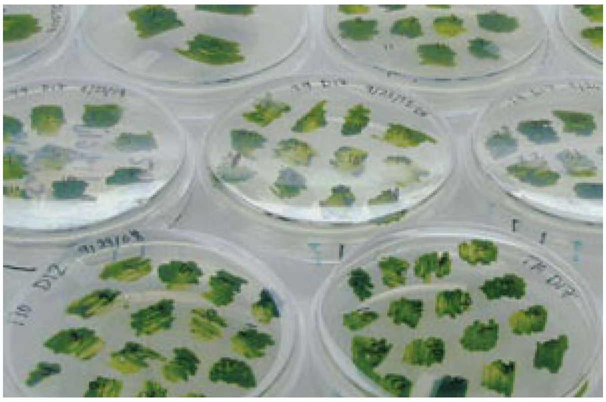
When Polle does find algae that show promise as biofuel producers, he passes them along to his collaborator, Christoph Benning, a plant biochemist at Michigan State University. Benning performs genetic experiments to uncover the biochemical mechanisms that make one algal strain more proficient than another at rapid growth and efficient oil production.

Benning, who's also funded through the USAF jet fuel program, admits that biologists lack a full understanding of the metabolic pathways algae use to produce oil. "We're missing the basic tools," he says. In algae, Benning explains, oils accumulate under physiologically stressful conditions, such as a lack of nitrogen or grossly fluctuating temperatures, which are counterproductive to vigorous growth. That's a central problem from the perspective of someone who wants to grow a healthy algal population that is also proficient at producing and storing oil. Through genetic experiments on *Arabidopsis* and on the lab rat of algae, genus *Chlamydomonas* (which naturally inhabits soils and is easily cultured in the lab), Benning hopes to identify transcription factors that are triggered by stressful environments, and could be used to encourage increased oil production in algae without slowing their growth from stress. "We're trying to identify the nuts and bolts of making oil in algae," he says.

Benning's lab has already produced promising results. In 2005, Benning uncovered some of the genes and enzymes important to lipid biosynthesis in *Chlamydomonas reinhardtii*, isolating *BTA1Cr*, a gene responsible for producing a critical membrane lipid in the species (*Euk Cell*, 4:242-52, 2005). Since *Chlamydomonas* is not an ideal oil producer, Benning hopes that what he learns in this model system translates into other, more biofuel-friendly species that Polle might turn up.

"We're investors in science sometimes, technology all the time, and magic infrequently," says Erik Straser, leader of the Cleantech team at Mohr Davidow Ventures, a Silicon Valley venture capital firm. Straser's investment portfolio includes a company that feeds farmed pine and poplar trees to bacteria that normally inhabit termite guts and produce ethanol. While scientists are working to develop the technological tools necessary to make biofuels from genetically modified organisms commercially viable, Straser says existing biofuel companies are scrambling to scale up their operations to sizes that will make a real dent in US fuel consumption. Scaling is the magic that Straser awaits. "It's a lot harder than people think."

For a biofuel company to make serious headway in the US fuel market, it must prove that it can produce at least one million gallons of fuel per day, according to Straser. And accomplishing that, he says, takes some major machinery. "You're going to need [closed-tank bioreactors] the size of a football stadium." And biofuel efforts based on open-pond growth of photosynthetic algae, which gather at the surface to draw energy from sunlight, might need considerably more space.



Algae samples await testing in Solazyme's lab.

Courtesy of Solazyme

Pat Gruber, CEO of Gevo, a company that produces the biofuels butanol and isobutanol using bacteria and yeast cells, says that going the photosynthetic route is a bit of a pipedream. "There's not enough freakin' land and water in the world to do that. What we've got here is a lot of emotion running rampant without facts being thrown on the table." Gevo's genetically altered "bugs" consume sugars in closed reactors and produce fuels similar, but superior, to ethanol, Gruber says.

Michael Borrus, founding general partner of X/Seed Capital, agrees that scale is a big hurdle. "The big problem with biofuels is that no one knows how to scale anything," he says. "It is possible, sure, but it's not an easy proposition."

One Israeli company, Seambiotic, maintains a 1,000-m² site with eight oblong ponds that can produce approximately 23g/m²/day of algae, according to its scientific advisor and algal growth expert Ami Ben-Amotz. That growth rate approaches US DOE's stated (but never reached) goal of 50g/m²/day. Ben-Amotz says that Seambiotic shipped approximately three tons of algae belonging to genus *Nannochloropsis* to biofuel manufacturers in 2008. But even this quantity of algal biomass does not yield one million gallons of biofuel per day-not even close. Ben-Amotz says that his algal cells typically contain 30% oil per gram of biomass, so 3 tons would only yield slightly more than 816,466 grams (or approximately 235 gallons) of algal oil, which could yield approximately 100-200 gallons of biofuel.

"I could ship 500 gallons tomorrow if someone wanted to buy it." - Harrison Dillon

With the help of seawater and free carbon dioxide from Israeli Electric Company smokestacks, Ben-Amotz says that Seambiotic's only limitation to increasing that growth rate is developing a better hydrodynamic system to churn the pond water more efficiently for proper aeration and increased algal growth rates. He's working with NASA on that one. "They got to the moon," Ben-Amotz says. "I hope they will solve the problem of water mixing!" Ben-Amotz says he thinks he can eventually achieve a growth rate of about 75g/m^2/day.

Next year Ben-Amotz says that Seambiotic expects to open a new open-pond facility-again sited at an electric plant-that will likely be the largest facility for algae production in the world. It will cover 5 hectares and will provide tons of algae to different production facilities; lipids will go to biodiesel manufacturers, sugars will go to bioethanol producers, and proteins to makers of nutraceuticals. But even Ben-Amotz admits that Israel doesn't have enough land to support truly commercial-scale algae production. He says that similar facilities will need to be constructed in other countries in South and North America for that to become reality.

Eric Jarvis, a senior scientist at the US DOE's National Renewable Energy Laboratory (NREL) in Golden, Col., worked on the agency's algae fuel exploration program, dubbed the Aquatic Species Program, before it was halted in 1996. Jarvis participated in a large-scale, year-long algal growth experiment in open ponds in the desert outside of Roswell, NM. He says that experiment taught him a lot about the prospect of growing algae for biofuel in such a way. "These are ecosystems, and you have to think of them in a bigger sense," he says. "It's not just a row crop, where you plant it and harvest it."

Jarvis adds that keeping algal strains confined to ponds (especially if they're genetically engineered) is just one of the challenges of outdoor algal farming. One must also be aware that alien algal strains will likely end up in open ponds, potentially throwing the system into disarray.

Al Darzins, a principal group manager at the NREL, says that the agency is currently focusing more on using living organisms to convert waste cellulose, such as that from corn stover or switch grass, into ethanol. Algae work was virtually nonexistent at NREL a few years ago, he adds, but now the agency currently devotes about $1 million of its budget to algae projects. "We should reopen the Aquatic Species Program," he says.

Darzins says that the scientists working on the Aquatic Species Program were the first to clone the gene for Acetyl CoA Carboxylase (ACCase), an enzyme that functions in lipid production, and insert that gene into the algae *Cyclotella cryptica*. The close-out report published by the DOE after the conclusion of the program, which many in the algal oil business refer to as "The Algal Bible," identified the ACCase gene as a key player in algae's oil synthesis. With the gene in hand, researchers working on the program even patented it and managed to coax algal cells into over-expressing ACCase. "These early experiments did not, however, demonstrate increased oil production in the cells," the report reads. Says Darzins, "It was a good shot in the dark, but it was a shot in the dark, nonetheless."

I'm behind the wheel of a white Jeep Liberty Diesel, driving around the broad streets of South San Francisco. A colorful corporate logo blares "Solazyme" across the side of the vehicle. The car feels like any other diesel car or truck. Turn the key, the engine rumbles to life and the motor growls under the hood. But this Jeep is different. In the gas tank is a fuel produced completely by genetically engineered algae: A pure biofuel. Riding shotgun is Harrison Dillon, a microbial geneticist who is now president, chief technology officer, and cofounder of biofuel company Solazyme. "When we drive this thing down the street in downtown San Francisco, people cheer," says Dillon with a wide grin.

Dillon started Solazyme with some colleagues in 2003 ("when oil was cheap," he says), and kept a culture collection of a couple hundred *Chlamydomonas* strains in his own low-tech facility. "We bought the growth media, sterilized it in my kitchen, and stored it in the garage," he remembers.

Cargill's evaporation ponds concentrate salt and algae.

Courtesy of Cargill

They tried to grow the algae in outdoor ponds, but quickly realized that the productivity of the algae was nowhere near high enough to yield appreciable amounts of fuel. So they switched to heterotrophic species of algae, which directly consume carbon-based compounds rather than passively absorbing carbon dioxide from surrounding media. "That's when the technology just took off," Dillon recalls. "It really went exponential." The advantage of using heterotrophic algae, according to Dillon, is that they are bathed in their energy source; as opposed to photosynthetic species, which must jockey for a good sunbathing position among millions of their kin.

Story continues below

While Solazyme's exact species and strains of algae are a closely guarded secret, Dillon assures that the company uses several obscure strains as their workhorses. "You're lucky if you can get 10 papers to come up on PubMed that name them," he smiles. "We look at algae that have been isolated from all over the world," from Irish peat bogs to equatorial swamps. For feedstock, Solazyme's algae eat anything from waste glycerol and sugar cane to sugar beet pulp and molasses. "You can use just about anything," Dillon says, as long as the feedstock is high-volume and low-cost.

Dillon says that Solazyme's algae produce some hydrocarbons, but mostly triacylglycerides. To make their biodiesel, the company takes the glyceride backbones from these fats and adds methanol. To get renewable diesel, they take that fatty acid methyl ester and "hydrotreat" it, stripping off oxygens and saturating the molecule with hydrogens. That gives them a straight-chain alkane, not much different from the diesel that flows from gas pumps into millions of diesel engines everyday across America. In nature, algal cells are rarely above 30% oil. Yields of 50-60% oil per gram of dry weight of algal cells are considered excellent. Solazyme's algae, however, stores 75% oil per gram of dry weight. "We have incredibly good scientists here," Dillon says.

Solazyme is most strikingly different from its competitors for the fact that its organisms produce not just transportation fuels, but also other consumer products-a way to diversify their business and leverage high-cost goods against the low price bar set for fuels. On a table in the company's boardroom sit about 10 jars of cosmetic goops and nutriceutical concoctions. I tentatively dip the tip of my finger into what Dillon calls Solazyme's "olive oil," and bring it reluctantly to my lips. Though my mother's Italian ancestors would be rolling in their graves at calling this stuff olive oil, it was edible.

Dillon says that he expects Solazyme to be producing algal biofuel at "demonstration levels of tens to thousands of gallons" per day by 2009, and aims to be producing its fuel products at commercial levels by 2011. "The scalability is not something that frightens me too much," he says.

"A big basic strategy of ours is to fit into existing infrastructure at every step of the way." Using large-scale fermentation tanks that currently churn out a wide variety of microbially-produced products-nutriceuticals, amino acids (lysine) for animal feeds, carpet fibers, components of infant formula, and laundry detergent enzymes-Solazyme hopes to fill existing petroleum pipelines with their diesel, which can run in unmodified diesel engines. "We were the first company to walk into a major oil company conference room with a barrel of microbially-produced oil," Dillon crows. "I could ship 500 gallons tomorrow if someone wanted to buy it."

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