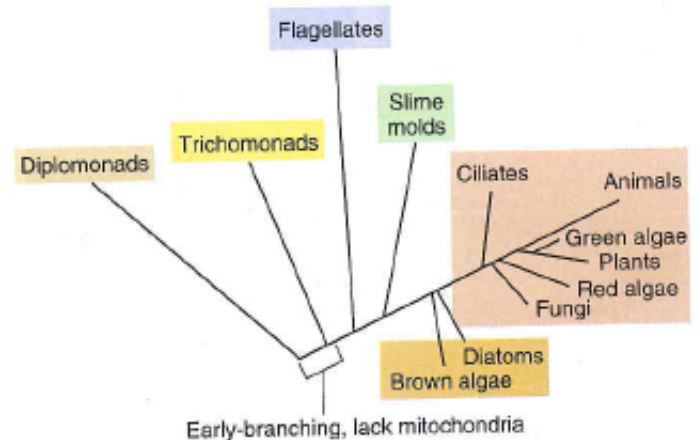


2.6 Eukaryotic Microorganisms

Eukaryotic microorganisms are related by their distinct cell structure (Figure 2.1) and phylogenetic history (Figure 2.7). Inspection of the domain *Eukarya* (Figure 2.22●) shows a long branch in the tree of life culminating in the most derived **eukaryotes**, the plants and animals. Interestingly, however, and consistent with their phylogenetic location on the tree, some of the “early-branching” *Eukarya* turn out to be structurally simple eukaryotes, lacking mitochondria and some other key organelles. These cells, such as the diplomonad *Giardia* (Figure 2.22), appear to be modern descendants of primitive eukaryotic cells that did not engage in endosymbiosis or perhaps had, but then lost their endosymbionts (Sections 2.3, 11.3, and 14.4). Most of these early eukaryotes are parasites of humans and other animals, and are unable to live a free and independent existence.

Eukaryotic Microbial Diversity

Like prokaryotes, a diverse array of eukaryotic microorganisms is known. Collectively, microbial eukaryotes are known as the **Protista**, or *protists* for short. Some protists, such as the algae (Figure 2.23a●), are phototrophic. Algae contain chlorophyll-rich organelles called *chloroplasts* and can live in environments containing only a few minerals (for example, K, P, Mg, N, S), H₂O, CO₂, and light.



● **Figure 2.22 Detailed phylogenetic tree of Eukarya.** Not all known lineages of *Eukarya* are depicted. Some early-branching species of *Eukarya* lack organelles other than the nucleus. Note how “higher organisms” (plants and animals) branch near the apex of the tree.

Algae inhabit both soil and aquatic habitats and are major primary producers in nature. Fungi (Figure 2.23b) lack photosynthetic pigments and are either unicellular (yeasts) or filamentous (molds). Fungi are major agents of biodegradation in nature and recycle much of the organic matter in soils and other ecosystems.

Cells of algae and fungi have cell walls, whereas the protozoa (Figure 2.23c) do not. Most protozoans

What are Biofuels from U.S. Environmental Protection Agency (EPA). 2013. From: <http://www.epa.gov/ncea/biofuels/basicinfo.htm>.

What are Biofuels?

Biofuels are fuels derived from renewable biological materials such as ethanol from corn kernels, corn stover, perennial grasses, woody biomass, and algae, and diesel from soy beans. Currently available biofuels are made from sugar crops (sugarcane, sugarbeet), starch crops (corn, potatoes), oilseed crops (soybean, sunflower, rapeseed), and animal fats. Sugar and starch crops are converted through a fermentation process to form bioalcohols, including ethanol, butanol, and propanol. Oils and animal fats can be processed into biodiesel. Ethanol is the most widely used bioalcohol fuel. Most vehicles can use gasoline-ethanol blends containing up to 10% ethanol (by volume). Flexible fuel vehicles can use gasoline-ethanol blends containing up to 85% ethanol. Currently there are only about 700 fueling stations in the U.S. that offer E-85 fuel, most of which are in the upper Midwest.

[Source: [SmartWay Biodiesel Fact Sheet](#)]

Second generation biofuels, or cellulosic biofuels, are made from cellulose, which is available from non-food crops and waste biomass such as corn stover, corncobs, straw, wood, and wood byproducts. Third generation biofuels use algae as a feedstock. Second and third generation biofuels are not yet produced commercially.



Algal Biofuels

Biofuels made from microalgae hold the potential to solve many of the sustainability challenges facing other biofuels today.

Algal biofuels are generating considerable interest around the world. They may represent a sustainable pathway for helping to meet the U.S. biofuel production targets set by the Energy Independence and Security Act of 2007.

Microalgae are single-cell, photosynthetic organisms known for their rapid growth and high energy content. Some algal strains are capable of doubling their mass several times per day. In some cases, more than half of that mass consists of lipids or triacylglycerides—the same material found in vegetable oils. These bio-oils can be used to produce such advanced biofuels as biodiesel, green diesel, green gasoline, and green jet fuel.

Renewed Interest and Funding
Higher oil prices and increased interest in energy security have stimulated new public and private investment in algal biofuels research. The Biomass Program is reviving its Aquatic Species Program at the National Renewable Energy Laboratory (NREL) to build on past successes and drive down the cost of large-scale algal biofuel production. NREL, Sandia, and other laboratories are also launching research into algal biofuels for private investors and programs within the Defense Advanced Research Projects Agency (DARPA) and Air Force Office of Scientific Research (AFOSR).



Benefits of Algal Biofuels

Impressive Productivity:

Microalgae, as distinct from seaweed or macroalgae, can potentially produce 100 times more oil per acre than soybeans—or any other terrestrial oil-producing crop.

Non-Competitive with Agriculture:

Algae can be cultivated in large open ponds or in closed photobioreactors located on non-arable land in a variety of climates (including deserts).

Flexible on Water Quality:

Many species of algae thrive in seawater, water from saline aquifers, or even wastewater from treatment plants.

Mitigation of CO₂:

During photosynthesis, algae use solar energy to fix carbon dioxide (CO₂) into biomass, so the water used to cultivate algae must be enriched with CO₂. This requirement offers an opportunity to make productive use of the CO₂ from power plants, biofuel facilities, and other sources.

Broad Product Portfolio:

The lipids produced by algae can be used to produce a range of biofuels, and the remaining biomass residue has a variety of useful applications:

- combust to generate heat
- use in anaerobic digesters to produce methane
- use as a fermentation feedstock in the production of ethanol
- use in value-added byproducts, such as animal feed

Challenges to Commercialization

Algal biofuels are not economical to produce using the technology available today. Based on conservative estimates, algal biofuels produced in large volumes with current technology would cost more than \$8 per gallon (in contrast to \$4 per gallon for soybean oil today).

Lowering this cost will require coordinated R&D across a wide range of technical sectors (listed at right) over the next 5 to 10 years. Although the technical challenges are significant, the broad public benefit of successfully commercializing algal biofuels warrants placing a high priority on the needed research. Particular attention must be paid to the engineering of sustainable microalgal systems and to the regulatory and environmental landscape.

Next Steps

To identify and prioritize R&D needs along the critical path to commercialization, DOE is holding an Algal Biofuels Workshop in Washington, D.C., in December 2008.



NREL and Sandia National Laboratories are working with DOE to plan and conduct this workshop, which will provide input for development of an Algal Biofuels Roadmap.

The roadmap will draw upon the expertise of a carefully balanced group of invited scientists and other experts in the various required disciplines (e.g., biology, systems and process engineering, modeling and analysis, algae cultivation, algal oil extraction and conversion, algal-based co-products, water and land use, policy and regulatory issues, etc.). Input from workshop participants will help define activities needed to resolve uncertainties associated with commercial-scale algal biofuel production. Upon completion of review and concurrence cycles, the resulting roadmap will be made available to the general scientific community in 2009. For updates as this process unfolds, please watch for news on our website: www.biofuels.energy.gov

R&D Focus Areas for Algal Biofuels

Basic Algal Biology

- Algae strain isolation and screening
- Genetics, genomics, strain improvement tools
- Photosynthesis and solar conversion efficiency
- Algae lipid productivity; biochemistry, and regulation of lipid accumulation

Process Research

- Algae mass cultivation
- Control of competitors, grazers, and pathogens
- System design and engineering
- Algae for wastewater treatment.

Production and Integrated Process Scale Up

- Long term maintenance of desired strain in culture
- Hydrodynamics of mixing
- Evaluation of local water supply for algal cultivation
- CO₂ supply
- Harvesting technology
- Oil extraction technology
- Optimization of specific fuel production processes
- Analysis of algal biofuels for compliance with ASTM standards.

Economic Analysis

- Detailed process analysis
- Potential for value-added co-products
- Resource and siting analysis
- Environmental and social issues
- Environmental impact of large-scale algae farms
- Water usage and process water disposal
- Regulatory issues, especially cultivation of genetically modified algae
- Public awareness and acceptance

For additional information, please contact:

The EERE Information Center
(877) EERE-INF (337-3463)
www.eere.energy.gov/informationcenter

Visit our website at
www.biofuels.energy.gov

A Strong Energy Portfolio for a Strong America

Energy efficiency and clean, renewable energy will mean a stronger economy, a cleaner environment, and greater energy independence for America. Working with a wide array of state, community, industry, and university partners, the U.S. Department of Energy's Office of Energy Efficiency and Renewable Energy invests in a diverse portfolio of energy technologies.



U.S. Department of Energy Energy Efficiency and Renewable Energy

Bringing you a prosperous future where energy is clean, abundant, reliable, and affordable



Algae farms require vast water surface areas to efficiently convert sunlight into an oil used as a biofuel.

ALGAE

The scum solution

The green slime that covers ponds is an efficient factory for turning sunlight into fuel, but growing it on an industrial scale will take ingenuity.

BY NEIL SAVAGE

When you imagine the crops that will provide biofuels, what is the first image that enters your mind? A field of corn or sugar cane? Maybe you should be picturing pond scum instead.

Algae, the organisms that cover ponds with a green film and turn tides red, are a promising source of biofuels. Researchers estimate that algae could yield 61,000 litres per hectare, compared with 200 litres to 450 litres from crops such as soya and canola. And, as the price of petroleum soars, that sort of yield is drawing interest from government and industry alike. Last year, the US Department of Energy gave US\$44 million to create a research consortium to advance the technology for turning algae into fuel.

Industry is also gearing up. Sapphire Energy, a renewable energy company headquartered in San Diego, California, has received more than US\$100 million in private investments to develop 'green crude', as well as another US\$104 million from the US federal government's 2009 stimulus package. The oil company Exxon Mobil gave a US\$300 million vote of confidence to algae by teaming up with the biotechnology company Synthetic Genomics in La Jolla, California. And aircraft maker Boeing helped establish the Algal Biomass

Organization to promote the creation of algal jet fuel.

That algae draws so much research attention is a testament to its compelling potential. The organisms can be grown in artificial ponds on land that's unsuitable for agriculture, so they don't have to compete with food crops for space. They can be cultivated on the surfaces of lakes or coastal waterways, or in vats on wasteland. Algae reproduce rapidly, spreading over a body of water within hours. And they can thrive on what would otherwise be considered a clean-up problem — the water from waste-treatment plants and the carbon dioxide spewed from industrial chimneys.

Most algae being explored for biofuel production are single-celled organisms that turn carbon dioxide, hydrogen and nitrogen into carbohydrates, lipids and proteins. Depriving the organisms of nutrients causes the photosynthetic mechanism to switch from growing more algae to producing lipids. After a few days, a centrifuge is used to separate the algae from the water they grow in. Breaking open the cells then allows the extraction of an oil that can be turned

into a hydrocarbon-based fuel. The alga's protein and carbohydrate remnants can be processed into pharmaceuticals or used as animal feed.

But what's simple to describe can be difficult to accomplish efficiently. Just ask GreenFuel Technologies, a company founded in 2001 by researchers at the Massachusetts Institute of Technology (MIT) in Cambridge. GreenFuel built a series of ever-larger pilot plants that used waste gases from power plants as a food source for oil-producing algae, and it signed a US\$92 million deal to build more plants in Spain. In 2009, the company shut down because of a lack of funds, having learned that harvesting algae was more expensive than it had anticipated. A recent study of algal biofuel production by the Energy Biosciences Institute at the University of California, Berkeley, which is funded by the oil company BP, found that much work remains before the struggle for economic viability can be won. According to Nigel Quinn, an agricultural engineer at Lawrence Berkeley National Laboratory who led the study, making fuel from algae using today's technology is a money-losing proposition, unless it's done in conjunction with another process, such as treating wastewater or producing valuable by-products.

To reach the big time, algal oil production must overcome several obstacles. For one thing, there's the question of space: for photosynthesis to work, light must reach the algae. If a layer of algae is more than a few centimetres thick, the organisms on the surface shade

➔ **NATURE.COM**
to read the latest
research on algae
biofuels
go.nature.com/WSJklr

those underneath, blocking the sunlight. One alternative is to spread horizontally — and wide. Algae would need to cover an area of 9.25 million hectares — about the size of Portugal — to derive enough biodiesel to cover Europe's annual transport requirement of 370 billion litres, according to René Wijffels and Maria Barbosa, environmental technologists at Wageningen University's Food and Biobased Research centre in the Netherlands.

Realistically, only 5.5% of land in the United States is available to accommodate algae-growing ponds, estimates Mark Wigmosta, a hydrologist at the Pacific Northwest National Laboratory in Richland, Washington. With the current technology, that land could produce 220 billion litres of algal oil per year — equivalent to about half of the oil imported by the United States for transport each year. Furthermore, with current production processes, such a large-scale algae-growing enterprise would require roughly three times as much water as is devoted to all US agriculture, says Wigmosta. To assess whether water usage could be reduced, he looked at areas where the average levels of sunshine, precipitation and humidity would lead to more efficient algal growth: the Gulf Coast, the southeastern seaboard and the Great Lakes. He found enough land in these regions to replace about 17% of petroleum imports with biofuel, using only one-quarter of the water devoted to agriculture (roughly the same amount of water that bioethanol production requires). Wigmosta based this analysis on a system using open ponds of 30 centimetres depth and 4 hectares in area, assuming they are supplied by fresh water. Strains of algae that grow in salt water or waste water could make the equation more favourable.

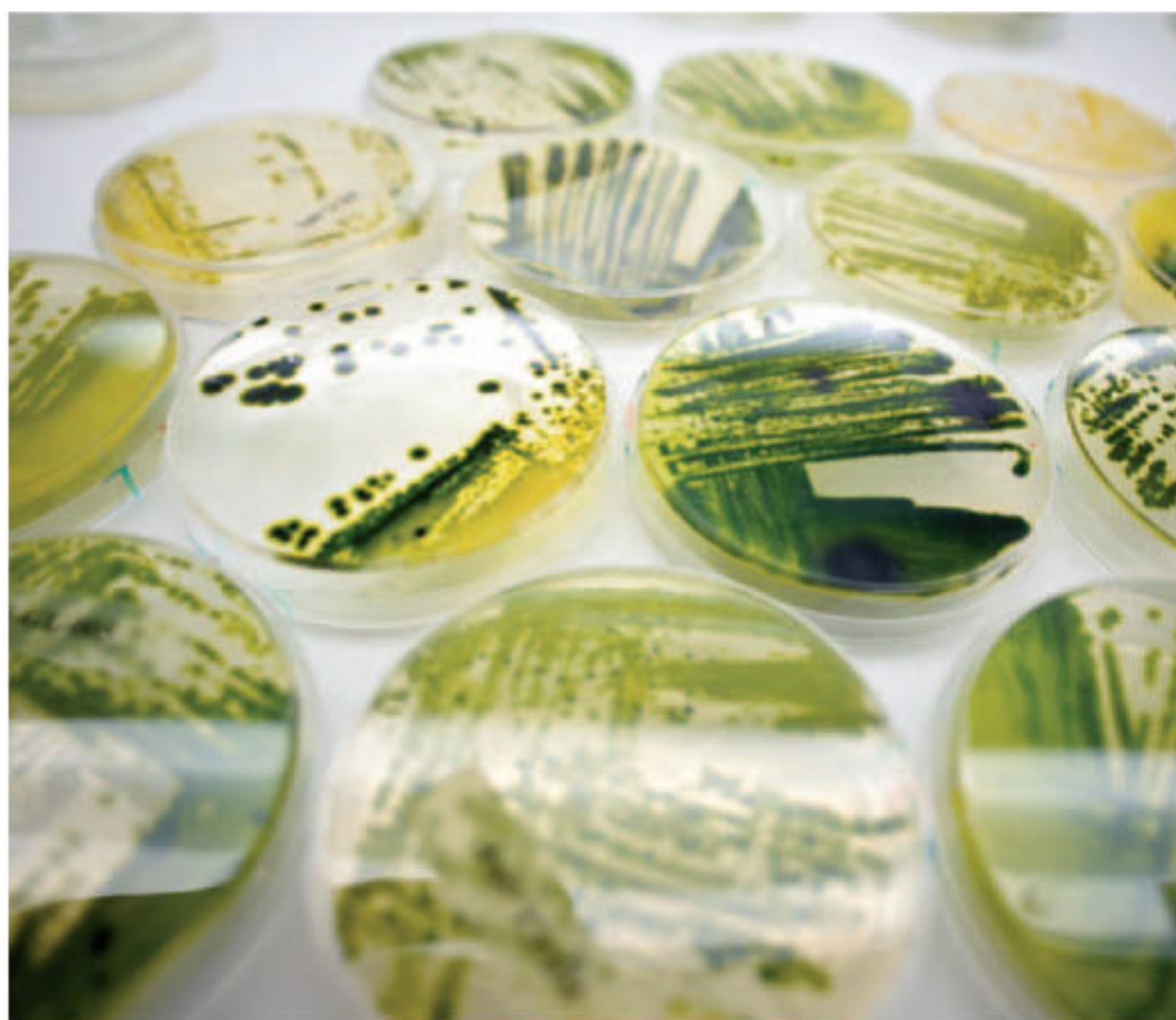
It might be possible to use less water by switching from open ponds, which lose water through evaporation, to closed photobioreactors. In a typical reactor, an array of glass tubes circulates CO₂ through a mixture of algae and water; the goal is to expose all organisms to enough sunlight. But such systems — which Wigmosta says are popular in China — present their own difficulties. For example, because the reactors soak up sunlight, they need to be cooled, which often means spraying them with water, possibly cancelling out the savings made by avoiding evaporative loss.

The other input that algae need, besides water, is CO₂ — but algal cells can't efficiently tap into atmospheric CO₂ to support the rapid growth needed for a commercial operation. So algal farms might need to be situated near artificial CO₂ sources, such as coal-burning

power plants. "If you have to pipe CO₂ four or five miles, the piping costs will eat you alive," Quinn says.

ADVANCED ALGAE

The renewable oils company Solazyme in South San Francisco, California, is trying to sidestep some of the problems with algal cultivation by exchanging photosynthesis for the same sort of fermentation used to produce ethanol. "The productivity is incredibly low when you grow algae in a direct photosynthesis process," says Solazyme president and chief technology officer Harrison Dillon. The company keeps its algae in the dark and feeds them sugar, which can be derived from any source. The organisms then convert the sugar into an oil. Dillon predicts that even without government subsidies, the company's biofuel will be priced competitively with petrol (gasoline).



Algae at Solazyme are kept in the dark and fed sugar to produce an oil.

line). Solazyme is converting old factories to demonstrate its technology and has a contract to deliver 570,000 litres of alga-derived fuel to the US Department of Defense this year. The company hopes to be selling algal oil to commercial refineries to produce a hydrocarbon-based fuel by the end of 2013.

James Liao, a chemical and biomolecular engineer at the University of California, Los Angeles, also wants to move away from traditional ways of using algae. The main issue with restricting nutrients to force the organisms to make oil is that it trades off growth for oil production. Liao, instead, adds more nutrients. The result is an artificial algal bloom, which yields little oil but a lot of protein. The algae then become a feedstock for another organism, such as *Escherichia coli*, which digests the algae and produces alcohols such as ethanol and butanol. Those, in turn, can be built up into hydrocarbon-based fuels by using standard chemical processes. One advantage of Liao's

proposal is its efficiency. "It's probably the fastest way to fix CO₂," Liao says. Another is that it avoids one potential problem of open ponds, the invasion of other organisms. Strains that have been genetically engineered to produce more oil may have trouble out-competing natural strains that enter the system. As a bonus, the conversion process produces ammonia as a by-product, and this nitrogen source can be used to fertilize the next round of growth.

Another possible fuel source is blue-green algae, which aren't strictly algae but bacteria of the genus *Cyanobacterium*. Whereas algal cells must be destroyed to extract their oil, cyanobacteria secrete their products. As a result, it's unnecessary to kill one batch and grow a new one, allowing continuous production. George Church, a geneticist at Harvard Medical School in Boston, Massachusetts, has engineered cyanobacteria to produce hydrocarbon molecules at the appropriate lengths for various fuels. "We're not making oils. We're making something much closer to petroleum," says Church, who cofounded Joule Unlimited in Cambridge, Massachusetts, to commercialize the technology. Moreover, Church says, tweaking the bacterium's genes will eventually make it possible for the organisms to soak up atmospheric CO₂ efficiently — an advance that would liberate fuel production from the need for an artificial source of CO₂. The company is testing its technology in a pilot plant near Austin, Texas, and expects to begin commercial production in 2012. The Joule team contends that its process will produce 140,000 litres per hectare per year.

There is plenty of room for progress. Current estimates are that biological photosynthesis can convert at most about 10% of the sunlight reaching the Earth's surface to chemical energy; Wigmosta says today's algae convert about 1.1%. Genetic engineering could create algae that produce more oil and are more efficient at converting solar energy to biomass. Engineers are working on improved designs for growth systems, such as structures that stack algae in layers for better sunlight exposure, and harvesting systems that could use microwaves or sound waves to extract the oil.

Quinn says it could easily take ten years of such research to make algal biofuels economically viable, but it's certainly possible to replace some proportion of the petroleum we use now. "We don't necessarily know what the path's going to be," he says. But "we're optimistic". ■

Neil Savage is a science writer based in Lowell, Massachusetts.

SOLAZYME, INC.