Algal Biofuels: THE RESEARCH

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The individual technical elements of the algae-to-biofuels process have already been demonstrated at the laboratory scale. Ongoing research seeks to refine the technology to enable scaleup to commercial production.

Although the large-scale production of biofuels from algae faces significant challenges that require further research, algal biomass does offer many advantages for biofuel production. Productivities per acre are higher than those of typical terrestrial forage and oilseed crops. Algae are a non-food-based biomass resource that can be cultivated on nonarable land, eliminating competition with agricultural crops for the same land. Algae can consume water from a wide variety of nonpotable sources, such as brackish, saline, marine, and wastewater. And the production of valuable co-products, such as biopolymers, proteins, polysaccharides, and pigments, increases the net present value of the process *(1)*.

Laboratory experiments have demonstrated that some microalgae can accumulate significant amounts of lipids — more than 50% of their dry cell weight. However, field data do not typically support these findings. Thus, there is still a need to develop a diverse technological portfolio to increase both algal biomass and lipid production and determine better methods for scaling up production. This article summarizes the state of academic, government, and industrial research and discusses technoeconomic challenges that must be overcome before algal biofuel can be produced sustainably (both economically and environmentally).

Microalgae

Microalgae are microscopic unicellular species, ranging in size from a few microns to hundreds of microns, that exist individually, or in chains or flocs, in freshwater and marine systems. Microalgae contribute approximately 40–50% of the oxygen in the atmosphere and simultaneously consume carbon dioxide to grow photoautotrophically without additional carbon sources. It has been estimated that about 200,000–800,000 species exist, of which about 35,000 species are already described in the literature. More than 15,000 compounds originating from algal biomass have been chemically identified *(2)*.

Some species of microalgae are cultivated and sold as food supplements or nutriceuticals. For example, the blue-green algae *Spirulina* sp. has a high protein content and is a source of omega-3 alpha-linolenic acid. Other species are being cultivated in pilot-scale facilities and studied as a potential feedstock for making biopolymers, proteins, polysaccharides, pigments, and biofuels.

The production of biofuels from microalgae (Figure 1) is currently receiving considerable attention. Microalgae can be converted into several different types of renewable (naturally replenished) biofuels, including green diesel, jet fuel, methane (biogas), ethanol, and butanol, as well as a variety of co-products. However, significant technological challenges must be overcome before algal biofuels will be able to compete economically with petroleum-based fuels.

Algal strain screening and genetic manipulation

Maximizing lipid production at the molecular level involves a thorough understanding of the metabolic pathways, especially those associated with lipid synthesis, and with packaging (*i.e.,* the storage of lipid material) and secretion. Although it is unclear whether regulations will prohibit or restrict the production of biofuels from genetically modified algal species, fundamental biological techniques such as genomics, proteomics, and transcriptomics must be applied to algal systems to gain insight into algal biology.

On the most fundamental level, species including

Nannochloropsis and *Botryococcus* are being sequenced. Promoters, such as the Rubisco promoter, are being evaluated for controlling genetic expression. In addition to algal vectors, universal plant vectors such as the IL60 vector and fungal vectors are being tested to determine whether they can be used to transfer genes from one algal species to another.

Algae must be able to survive in the presence of other organisms (bacteria, diatoms, etc.) in nonsterile environments, while maximizing the production of lipids. Therefore, crop protection studies involving adaptive evolution and genetic modification of algae to provide a competitive growth advantage are underway. For example, some algal species are tolerant to pesticides and others are resistant to antibiotics. Some species produce long-chain hydrocarbons (C20 to C30) and grow in clumps but grow very slowly, such as *Botryocucuss* sp., while others like *Nannochloropsis* sp. make shorter-chain (C14 to C18) unsaturated fatty acids and grow relatively quickly as individual cells in salt water.

Ideally, an algal strain that grows rapidly over a range of temperatures while producing hydrocarbons of a specified chain length (such as C12) that can be cultivated in low-quality water with variable salinity or wastewater is desirable. Other desirable traits may include the ability to secrete lipids or to auto-flocculate, and the ability to grow in minimal light. The ideal strain does not yet exist, but biologists are mining the natural diversity of algal strains, as well as adaptively evolving and transforming algae to confer desired traits to maximize productivity and photosynthetic efficiency. Rapid screening techniques are being developed to examine the vast diversity of species for strains that produce more lipid, synthesize lipids with shorter chains (more C12 hydrocarbons, which are ideal for fuel), produce larger quantities of biomass, and grow efficiently in a variety of nonpotable water sources.

Algal cultivation

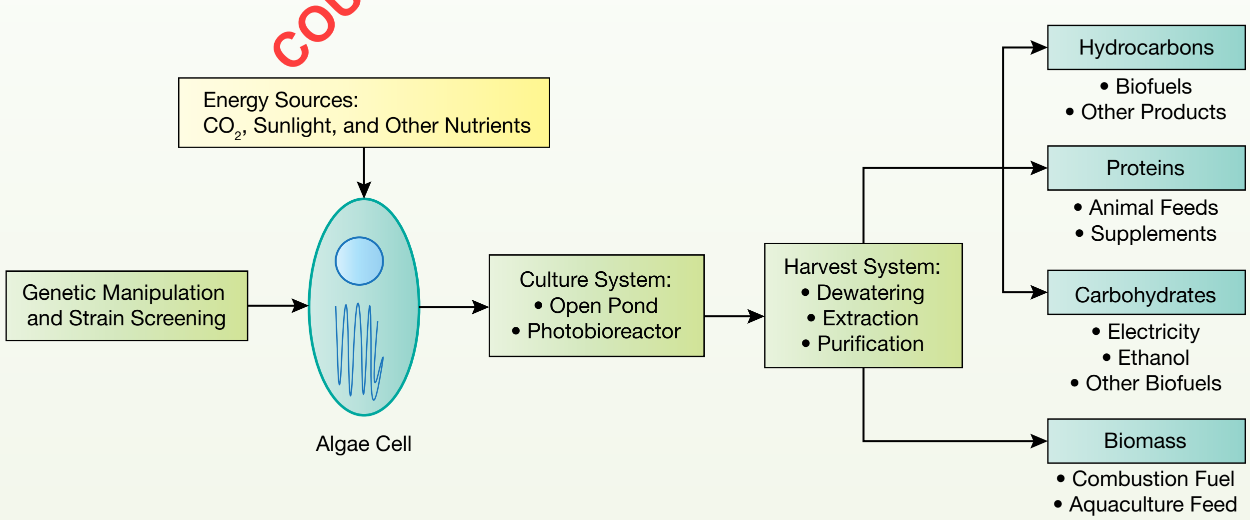
Significant research has been conducted on establishing the optimal nutritional composition and culture conditions for hydrocarbon production by microalgae.

The production of hydrocarbons in microalgae cells is growth-associated and usually reaches its maximum around the early stationary phase (the latter phase) of the algal growth cycle. Once in the stationary phase, algae tend to switch from making membrane lipids to making triacylglycerides. Many factors are known to affect hydrocarbon accumulation and fatty acid composition, such as nutrient compounds (especially N and P), temperature, light intensity and duration, pH, minerals, and even the growth cycle itself. Some strains of microalgae accumulate lipids only under stress conditions *(3)*. For example, *B. braunii* can accumulate more C18:1 under nitrogen deficiency and salt stress.

There are four major cultivation conditions for microalgae: photoautotrophic, heterotrophic, mixtrophic, and photoheterotrophic cultivation.

The most common way to cultivate algae is photoautotrophically, with CO_2 the sole carbon source and sunlight the energy source. The biomass productivity is usually low compared with the other methods, since energy (in the form of adenosine triphosphate, ATP) is required to convert CO_2 to biomass and nitrogen-limiting or nutrient-limiting conditions are usually applied to increase the lipid content in the cells. However, contamination is usually less severe.

Some microalgae can grow heterotrophically by consuming organic carbon (glucose, glycerol, fructose, sucrose,



▲ **Figure 1.** The sustainable, large-scale production of biofuels and other products from microalgae will require research in many aspects of the process.

galactose, mannose, and/or acetate) without light. Higher biomass production and productivity can be obtained under these conditions. For example, a lipid content of 58% has been reported for *Chlorella protothecoides* grown heterotrophically *(4)*, which is about three times higher than the lipid content of autotrophic cultures *(5)*. However, contamination is an issue during heterotrophic cultivation because bacteria thrive on organic carbon.

Another area of active research is the combination of algal cultivation and wastewater treatment, since wastewaters are rich sources of carbon, nitrogen, and phosphorus nutrients. The use of wastewater eliminates the need to add potentially expensive and scarce nutrients like phosphate.

Photobioreactor vs. open pond cultivation

Currently, researchers disagree about whether algae should be grown in closed photobioreactors, which are costly but less susceptible to contamination and easier to control, or in open ponds, which are considerably less expensive but challenging to operate year-round due to climate variations. Ongoing work is investigating the growth rate, productivity, fluid mixing, and light penetration for both reactor types, with the goal being to optimize the systems.

It is still too early to determine whether closed or open systems will ultimately be selected for commercial algae cultivation, so it seems prudent to support cultivation research and development projects that include ongoing life-cycle analysis (LCA) research. While it is true that capital costs for photobioreactor construction are currently higher than for open ponds, it is important to acknowledge the advantages and disadvantages of both systems. Traditionally, photobioreactors have suffered from problems of scalability, especially in terms of mixing and gas exchange (both CO_2 and O_2), and they require periodic cleaning to remove cell deposition from the walls. Although closed systems lose much less water than the open ponds, where evaporation is a factor, they do not benefit from evaporative cooling, so temperature maintenance can be a problem; open systems, on the other hand, require the continuous addition of water. Photobioreactors can also provide a higher surface-area-to-volume ratio than open ponds, allowing for better light penetration, resulting in higher volumetric cell densities, and reducing the amount of water for both culture maintenance and downstream harvest.

▲ **Figure 2.** The patent-pending design of the Univ. of Arizona ARID raceway allows for temperature control without heating or refrigeration. Cultures have been maintained even when temperatures dropped to 20°F.

Many of the disadvantages of photobioreactors are being solved through improved materials — *e.g.,* inexpensive plastic bags instead of heavy glass tubes — and improved engineering designs — *e.g.,* continuous or high-biomass cultivation. Open pond research is focusing on designs with inexpensive removable covers, better mixing strategies for improved control and light penetration, as well as new engineering designs such as the Arid Raceway Integrated Design (ARID) system at the Univ. of Arizona, shown in Figure 2. Methods for controlling invasive species and contamination are also being investigated.

Although LCA results for both photobioreactor and open pond systems have been published and presented, much of the information used is either out of date, based on assumptions instead of actual algal cultivation data, or based on proprietary information that is difficult to verify. As a result, it remains to be seen which system will be superior at production scale over long periods of operation. Currently, Sapphire Energy operates an open system in New Mexico, Solix Biofuels Inc. operates a closed system in Colorado, and Phycal and HR BioPetroleum are building/operating combined systems (photobioreactors and ponds) in Hawaii. There seems to be general agreement that photobioreactors will play a critical role as breeder/feeder systems linked to open raceways, providing high-cell-density uni-algal inoculum for production ponds *(6)* or a series of linked turbidostats or chemostats *(7)*.

The source of water and nutrients such as CO_2 is also of interest to the algal biofuels research community. Various algal species grow in wastewater, and many wastewater treatment facilities find algae problematic to their processes. Thus, the relationship between water quality and algal productivity is an active area of study.

Investigators are working to understand how trace metals, organics, nitrate, and phosphate influence algal growth and lipid yield, and whether bacteria, microalgae, and other species can be cultured together. The most-studied nutrient is CO_2, in particular whether fluegas can be used to cultivate microalgae. Preliminary experiments have claimed that this is reasonable, however, prolonged experiments using recycled water have not been published. Questions currently being examined are how to effectively capture the CO_2 from the fluegas, how to handle heat integration with the power plant, how to control the pH, what happens to the trace metals in the fluegas, and how tolerant microalgae are to SOx and NOx.

Harvesting and extraction

Cost-effective and energy-efficient harvesting and lipid extraction technologies need to be developed. Current bulk harvesting technologies include centrifugation, flocculation, gravity sedimentation, filtration, and screening. These methods require large amounts of energy and have high capital costs, since the effluent from an algal cultivation system contains only 1–2 g (dry weight) algae per L.

Hence, many groups are investigating harvesting technologies that use innovative strategies, such as acoustic focusing, hybrid capacitive deionization/electrophoresis and novel materials for traditional membranes and flocculent systems. Flotation, for example, is a gravity separation process in which air or gas bubbles attach to cells and carry them to the liquid surface, where the algae are removed by skimming *(8)*. Possible cost-effective flocculation methods include the use of the marine microalgae *Skeletonema* to co-bioflocculate *Nannochloropsis* or lime (which is also a calcium source for animal feed). Algal cells that are larger or have the ability to self-flocculate are also being investigated as a way to avoid the need for additional chemicals.

Electrophoresis, which removes the charged algae cells from the solution by applying an electric field, is another approach being evaluated to separate microalgae biomass without the need to add chemicals *(9)*. However, this technique is not yet mature and requires a more fundamental understanding of relationships between algal species and charge in order to improve the design.

After the dewatering process, lipids can be recovered from algal biomass by means of physical (mechanical disruption) processes, chemical (solvent extraction) processes, or both. Chemical extraction, typically using a chloroform/methanol/water or hexane solvent system to extract dried samples, is more effective and preferred on a laboratory scale. However, because this method is difficult to scale, researchers are focusing on reducing the use of toxic and polluting solvents and environmental-friendly extraction methods.

New lipid-extraction technologies involve innovations in acoustics, sonication, mesoporous nanomaterials, and amphiphilic solvents. For example, SRS Inc. designs solvent systems specifically for a given algae strain and has a test facility in New Mexico. Supercritical fluids (methanol or CO_2) are being evaluated for lipid extraction, and supercritical fluid extraction (SFE) has been reported to be safer and faster than traditional hexane extraction *(10)*. A team is testing a switchable solvent system, which can reversibly convert from a polar to a nonpolar form upon the addition of CO_2 followed by N_2 *(11)*. Another technique under evaluation is a milking system that manipulates the hydrophobicity of the solvent, allowing extraction of lipid from algal cells while maintaining their viability (rather than destroying the cells in the process). If successful, this method will offer the possibility of lipid selection during extraction *(1)*. The final but extremely important area of extraction system research involves developing a solvent that is reusable or recyclable to assure a sustainable process.

Conversion of algal oil to fuel

The most common type of algal extracts under consideration are lipids (*e.g.*, triacylglycerides), which can easily be converted into biodiesel. Chemical transesterification, enzymatic conversion, and catalytic cracking are all feasible processes for lipid conversion.

Transesterification, which is a relatively mature method, is performed via catalytic or noncatalytic reactions. A significant difference between converting petroleum to diesel and algal oil to biodiesel is that crude algal oil may contain high levels of phosphorous from phospholipids on the membrane, nitrogen from extracted proteins, and metals from chlorophyll. Thus, it is necessary to optimize both the level of purification of algal lipids and the tolerance of the catalyst for the contaminants to achieve the most cost-effective process.

In addition to traditional acid or base catalysts, solid catalysts have been tested at the bench scale. For example, Catalin, Inc. has developed a solid catalyst that produces high-purity glycerol in addition to biodiesel. Enzymatic systems are being studied but these are not yet cost-effective.

Finally, different types of mixing and heating systems are being evaluated. One that enhances the kinetics of transesterification involves the use of microwaves. Another is an ultrasonic reactor method, in which ultrasonic waves constantly produce and collapse bubbles in the reaction mixture, providing mixing and heating simultaneously.

Other fuels can also be produced from algae. Behind biodiesel, jet fuel has received the most attention. UOP has converted a mixture of bio-oils (including oil from algae) to jet fuel and tested the fuel in airplanes. Other areas of research, primarily by government laboratories and universities, include: gasification of algal biomass or lipid-extracted algae to syngas and/or methane; biological conversion of algal carbohydrate to ethanol or butanol; and catalytic conversion to other fuels.

A final challenge is that all of the biofuels must meet a multitude of performance specifications that include volatility, initial and final boiling point, autoignition characteristics, flashpoint, and cloud point. Thus, systems analysis of the processes to make these fuels is another current research focus.

Coproducts

Few industries or large companies can survive over the long term by producing and selling only one product. Furthermore, a barrel of oil is used for more than fuel. Thus, processes (reactors and separators) to make coproducts, including feedstocks for the chemicals, fertilizer, and specialty chemicals industries, are being studied.

Many of the agricultural universities are investigating the use of lipid-extracted algae (LEA) as an animal feed or supplement. Researchers are working toward understanding the nutritional content of LEA and developing processes that will lead to the certification of algal animal feed blends. The advantage of using the LEA as feed is that the production scales are on the same order of magnitude — the world requires large quantities of fuel that can be produced from the oil fraction of the algae and large quantities of food that can be supplied from the protein and carbohydrate fractions. (However, replacing all fuel with algal biofuels would leave an excess of LEA.)

Studies in which LEA serves as the source of nitrogen and phosphorous for fertilizer for terrestrial crops are underway in greenhouses. One probable use of the LEA is to recycle it to the algae cultivation system as a source of nitrogen and phosphorous. Cultivation studies using recycled nutrients along with recycled water are actively being pursued by many researchers.

Process, economic and sustainability modeling

Modeling tools, including ASPEN and CHEMCAD, have aided chemical engineers for decades in designing and scaling chemical processes, but they are used to a lesser degree for biofuels. A major shortcoming is the lack of a robust thermophysical-property database for the fatty acids, the resulting fuels, and the byproducts. Some companies use simulation tools with proprietary databases, but the information is not available for general use. Thus, characterization of lipids and fuels is an active area for government and university research.

The ultimate goal of algal biofuel research is to supply algal oil at \$2/gal that can be processed to fuel for approximately \$0.40/gal. Economic modeling is very important to realizing this goal.

Multiple economic models are available to calculate such quantities as energy return on investment and parasitic energy loss. However, the models do not produce consistent results — which makes it challenging to compare model output. Model inputs include: plant location; transportation, process, and equipment costs; feedstock costs (CO_2, water, trace nutrients); co-location and integration opportunities (power plants, wastewater treatment facilities, existing petrochemical facilities); and energy, co-product and fuel market variability. The ideal model output will include information useful in determining the best size and location for an algal biofuel reactor and processing facility, and whether small distributed units or large integrated facilities for algal growth, dewatering and extraction are better.

The National Alliance for Advanced Biofuels and Bioproducts (NAABB) consortium is developing an integrated model that will include economic and process information as well as siting and sustainability data. One part of the model is a comprehensive geographic information system (GIS) siting model. The inputs include available land (not currently being used for farming), grade or slope of the land, and water availability. The output is a map of the U.S. showing potential sites for algal cultivation.

There are also multiple lifecycle assessment (LCA) models in the literature for fuel production from algae. These models differ in the amount of the information that is assumed, obtained from a database, and based on experiments. Sorting through the literature is challenging, but necessary.

Scale-up barriers

Technical and economic barriers are inherent difficulties in the scale up of algae cultivation from laboratory and pilot facilities to commercial operation. Nutrient supply and water treatment and recycling are technically trivial and inexpensive at small scales, but represent major technical and economic problems at commercial scales. Incorporation of algae cultivation processes into existing agricultural or municipal waste streams will lower nutrient costs but could introduce unacceptable contaminants, such as pathogens, chemical compounds, and heavy metals, into the algae culture *(12)*.

Research results on artificial pond ecology or pathology are rare, and work in these areas will be critically important for the development of strategies for cultivation risk mitigation and remediation. Achieving large-scale culture stability will require a combination of fundamental research and laborious, empirical, optimization research.

Finally, regulatory issues involving multiple agencies at both the federal and state levels need to be considered. In particular, procedures for environmental risk assessment and review/approval of genetically modified algae need to be established and standardized. Overall, water management, agricultural, and environmental issues related to algal cultivation and production of biofuels require coordination across agencies.

Beyond the general concerns mentioned previously, the following areas must also be addressed for economically viable, sustainable, commercial-scale algal cultivation and production *(1)*:

- culture stability and susceptibility
- standardized operation procedures (SOPs) for system-level productivity analysis
- nutrient source scaling
- water composition, management, and recycling
- efficient dewatering and extraction techniques with solvent recycle
- heat integration
- economies of scale. CEP

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