REVIEW

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Exploiting diversity and synthetic biology for the production of algal biofuels

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Modern life is intimately linked to the availability of fossil fuels, which continue to meet the world's growing energy needs even though their use drives climate change, exhausts finite reserves and contributes to global political strife. Biofuels made from renewable resources could be a more sustainable alternative, particularly if sourced from organisms, such as algae, that can be farmed without using valuable arable land. Strain development and process engineering are needed to make algal biofuels practical and economically viable.

Despite limited supply and increasing demand, fossil fuels remain among the world's cheapest commodities. Prices will inevitably rise once demand starts to outstrip supply, but short- to medium-term replacement of fossil fuels by renewable and more environmentally benign alternatives will occur only if the substitutes can compete economically. One of these alternatives is based on the oils extracted from algae, and commercial-scale pilot facilities to test these are in operation. However, significant improvements are still needed to make algal biofuels economically viable. In this Review, we outline the advantages of algae as a biofuel producer, discuss the different cultivation methods, consider the options for achieving optimal algal biomass and lipid production, and the process engineering needed to make the process efficient and economically competitive.

Fuel from algae

Producing algal biofuel exploits the ability of algae to produce oils using only sunlight, carbon dioxide and water. Microalgae accumulate oil as nonpolar storage lipids, such as triacylglycerides (TAGs)[1]. The photosynthetic and cellular membranes of algae also contain polar lipids, such as glycolipids, phospholipids and sterols. Oils from algae can yield biodiesel through transesterification[2], and gasoline (petrol) or jet fuel through distillation and cracking[3].

Biofuel can be produced from various sources, but yield estimates are significantly higher for algae than for any other crop. This has considerable implications for land-area requirements: algae cultivated on only 30 million hectares and yielding biofuel at a conservative estimate of 40,000 litres per hectare per year is sufficient to replace the 1,200 billion litres of petroleum used by the world's largest consumer of petroleum, the United States (Fig. 1). The area is similar to that used for soya planting in the United States (about 29 million hectares) and roughly twice that used for the US production of corn ethanol (about 14 million hectares was used to produce almost 64 billion litres of ethanol in 2011), or an area about the size of New Mexico[4–6]. Furthermore, algal cultivation can use the large amount of non-arable land available for development without displacing food production, and its relatively high demand for water can be met by using low-quality sources such as waste or salt water[7].

Algae are almost ideal as organisms for developing the highly productive and robust crop strains that are essential for economically viable biofuel production. The search for these strains can exploit the vast diversity of algae, ranging from giant multicellular kelps to single-celled microalgae. Microalgae have been the focus of intense biofuel production efforts because of the ease, scale and speed at which they can be grown and manipulated, but strains differ significantly in lipid profile, photosynthetic ability, growth rate, growth medium requirement (from extreme halophilic to marine and fresh water), resistance to pathogens and biomass productivity. Although most algae are phototrophs, many can be grown heterotrophically.

Agricultural and industrial production

The underlying technologies and optimization strategies for producing biofuel through agricultural and industrial commercialization are very different.

In agricultural production, microalgae are cultivated in open ponds, with sunlight driving photosynthetic growth. This method uses non-arable land, consumes large amounts of CO_2 during the biomass production phase and, in principle, is extraordinarily scalable — limited only by space and capital costs. But growing algae efficiently and sustainably in fully exposed outdoor ponds is difficult, and suitable cultivation systems and practices are still under development.

Industrial production uses similar processes to industrial microbial fermentation in which yeast or bacteria are used as biorefining agents to produce food, beverages or high-value biotechnology products. For algae, production is driven by sunlight (in a photobioreactor) or reduced carbon sources such as sugar (in a fermentation reactor). Fermentation occurs in complete darkness and is identical to that of bacteria or yeast, which, as mature industrial technologies, have many of the systems and processes needed for algal fermentation already in place. However, fermentation has, so far, been used only to produce relatively high-value products; whether it can be developed for large-scale and economically viable fuel production is uncertain. In addition, algal fermentation of processed sugars to produce fuels heterotrophically does not produce lower greenhouse-gas emissions than the processing of fossil fuels[8], and is unlikely to equal their cost because the price of sugar is so high (more than US$0.66 per kilogram in 2011)[9]. Dependent on the development of advanced cellulose treatment technologies, using cellulosic biomass as the sugar source might be a solution[10]. However, this Review focuses on photobioreactors, which, like the agricultural process, rely on CO_2 and sunlight for photosynthetic production.

System optimization

Both agricultural and industrial production could be important in meeting the high demand for liquid fuels around the world. But their potential will be realized, and economic viability reached, only if the capital and energy costs of the production process can be greatly reduced and

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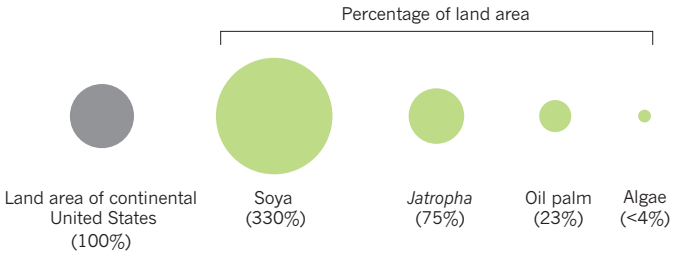

Figure 1 | Comparison of oleaginous crops. The United States consumes 25% of the world's petroleum. The land area needed to replace all domestic and imported petroleum used in the United States is shown as a percentage relative to the land area of the United States. The area required for algae is estimated to be significantly less than for any other biomass source[4,6].

the fuel yield enhanced.

The success of any biofuel production system will depend on economic and environmental costs. Life-cycle analysis (LCA) is a valuable tool for assessing this success, attempting to analyse the complete cost of a production process by considering the energy input and the environmental impact of each step. When combined with other economic analyses, LCA can help to identify high-cost areas, and determine whether a biofuel production strategy using a specific process and algal strain is viable. Also typically considered in an LCA, the basic processes involved in algal biofuel production, including the protection and maintenance of crops and the conversion of products into fuel (Fig. 2), are used to pinpoint where improvements will have a particularly strong affect on yield, environmental cost or energy use[11–13].

Not all LCAs take the same processes into account, which means the results can vary widely. For example, whether land-use change is considered[14] can be pivotal in determining whether a biofuel source reduces or increases greenhouse-gas emissions. Depending on the processes considered, annual yield estimates for agricultural algal biofuel production can vary between 8,000 l ha^{-1} and 140,000 l ha^{-1} (refs 15, 16). Overall output is of course important in determining the viability of a system, but even if the absolute numbers of LCA outputs vary, they still have a role in identifying the processes in need of optimization.

Unlike most agricultural systems, industrial production uses photobioreactors. These photobioreactors vary, but typically have a tubular or large, thin and flat panel design[17] (Fig. 3). Many factors can be adjusted to improve productivity and reduce costs, including culture media and nutrient sources, flow rates (amount of mixing) and sunlight exposure. Immobilized algal culture systems can be used for growth on a solid surface[17,18] to prevent shading and improve photosynthesis, which is especially useful for algae that secrete fuel precursors (Box 1), but early evaluation deemed this technology too expensive[19].

In a comparative LCA of the different growth systems, light-emitting diode (LED)-illuminated photobioreactors produced significantly more biomass (244,668 kg ha^{-1}) than solar-illuminated photobioreactors (8,262 kg ha^{-1}) and open ponds (4,957 kg ha^{-1}). But for the production costs per kilogram of biomass, this order was reversed: the estimated cost of production in open ponds was about $3 for a kilogram, five times lower than that achieved with solar-lit photobioreactors and eight times lower than with LED-lit photobioreactors[20]. So despite lower total biomass yield, the reduced input costs can result in a more efficient production system.

Irrespective of the organism used, the yield per input cost is the paramount factor for the control of overall economics. Improving algal growth while keeping input costs constant can neutralize many of the efficiency losses associated with the recovery of fuel. Yield is determined by the growth rate, cell density and lipid content of the algae, so optimization of each parameter affects this downstream processing efficiency. Higher growth rates reduce the time between harvesting, potentially reducing the exposure of algae to pathogens and the associated crop losses, and decreasing water consumption over a harvesting cycle. The final cell density of the culture — which determines the amount of biomass at harvest — is also important because harvesting is one of the more expensive processes. Engineering improvements will help to optimize efficiencies in biofuel processing, but the amount of biomass at harvest and its quality (lipid content) will affect productivity[21].

The yield of overall biomass and associated lipids that can be converted into fuel is controlled by a set of complex, and poorly understood, environmental and genetic factors. Achieving the best yield involves selecting promising strains (genotypes) and adjusting environmental factors, especially the growth-media composition. Although the relationship between metabolism and storage lipids is complex, understanding primary and secondary metabolism will allow strains to be engineered to suppress or remove processes that create energy-storing product molecules that are unsuitable for biofuel production. Conventional mutagenesis and genetic engineering have already been used to identify promising production strains, and recombinant DNA technologies are used to improve algal growth in both agricultural and industrial systems. Developing optimized production varieties will no doubt deliver profound yield improvements. Corn production today, for instance, has almost eight times the yield it did eight decades ago, before commercial varieties were developed[6].

Strain selection

Identifying the ideal algal strain for biofuel production is made easier by the immense diversity of the group, which has diverged over billions of years[22]. Algae have a potential genetic pool that is orders of magnitude larger than that of animals or land plants, but this diversity has only recently been examined. This potential is reflected in the diversity of algal species being explored for fuel production, which includes green algae, diatoms and cyanobacteria.

In agricultural systems, algal strains need to be able to tolerate variations in temperature, light, salinity and pathogen load. Strains optimized for cultivation in various geographical regions and in each season will be needed to ensure their widespread use. However, native strains are unlikely to have all of the characteristics needed for either agricultural or industrial production. No plant or animal used in large-scale agricultural or industrial production is the wild-type strain — they have all been domesticated by extensive modification through breeding and selection. A similar domestication process will be essential in obtaining the algal strains that generate adequate yield and quality of biomass.

Industrial bioreactors can be controlled, making them more stable environments than open ponds. Finding algal strains that grow quickly to high-cell densities and produce high levels of lipids using as little energy as possible will be essential to ensure cost-effective operations. Contamination is a significant factor in any cultivation system and, because stringent sterilization will almost certainly be too expensive, strains will need to have sufficient defence against or tolerance of pathogens to achieve high yields.

The temperature-control and gas-exchange systems needed in industrial systems to prevent alga death from excessive solar heating or oxygen poisoning are expensive components[23]. Most strains used in biofuel production grow best at 20–30 °C (ref. 23). Using thermophilic variants of cyanobacteria, which grow well between 35 °C and 50 °C, could be one solution to this expensive temperature control[24]. Solar heating is less of a concern with outdoor ponds, but lower growth during colder months — also applicable to outdoor photobioreactors — can significantly reduce yield. Locating ponds and outdoor photobioreactors in warm climates will compensate for these losses; however, ideally strains that produce high yields in a range of climates will be identified, or known strains will be modified. Many algae are already tolerant of cold temperatures and can recover from near freezing, but they grow slowly[25]. Several types of alga used for aquaculture are known to grow better at specific temperatures. For example, *Nannochloropsis* grows best at 25 °C; *Nannochloris* grows better at 30 °C; and *Chlorella vulgaris* has the best production at 10 °C (ref. 26). Rotation of algal strains based on growing season is therefore a likely production model, just as it is in terrestrial agriculture.

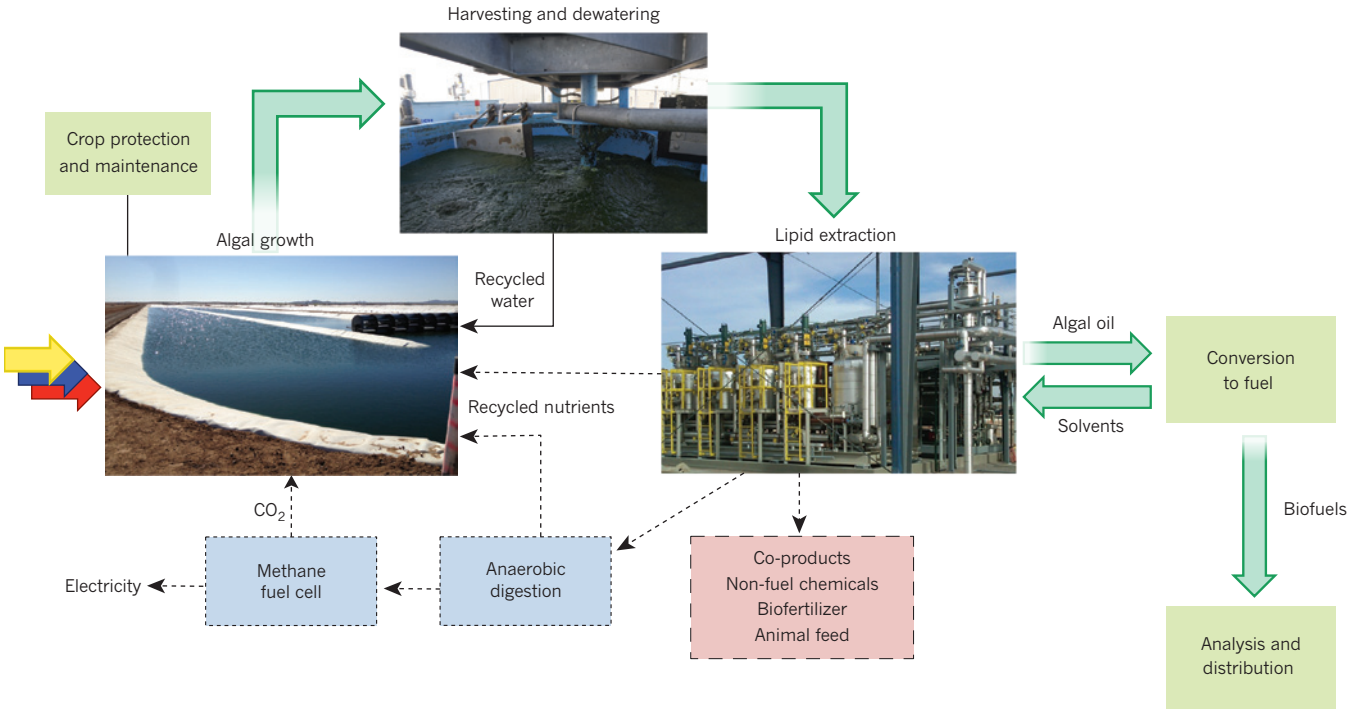

Figure 2 | Algal biofuel production. Light, water and nutrients (yellow, blue and red arrows) are required for algal growth in ponds. Some of the processes involved in algal biofuel production are common to most systems (green arrows). After fuel molecule extraction there are alternative uses for algal biomass (dashed arrows); many of these can produce co-products that are beneficial for economic and life-cycle analysis considerations. (Images courtesy of Sapphire Energy, San Diego, California).

A diverse set of wild-type strains have been isolated from different geographical and environmental sites, and evaluated for growth rate and storage-lipid accumulation[27–29]. Of 30 wild microalgal strains, *Nannochloropsis* sp. F&M-M26 was the most productive, achieving a lipid production of 61 mg l^{-1} per day through a biomass productivity of 0.21 g l^{-1} per day and a lipid content of 29.6% of the biomass[30]. The highest biomass productivity recorded is 0.37 g l^{-1} per day in *Porphyridium cruentum* (a red alga), and the highest lipid content is 39.8% in *Chaetoceros calcitrans* (a diatom); if these maximas were achievable in a single strain this could potentially be a 2.5-fold rise. Moving the most productive *Nannochloropsis* isolate to large photobioreactors increased lipid productivity to 84 mg l^{-1} per day. Despite this naturally occurring potential, the production efficiencies need to be much higher for commercial viability.

Strains of Chlorophyceae, Eustigmatophyceae, Haptophyceae and cyanobacteria are used to produce biofuels and other industrial organic chemicals[30], and many other types of algae have been tested. Although a natural isolate may exist that exceeds current yields, improving strains through breeding or recombinant DNA technologies is more likely to be successful in achieving the efficiencies needed for economic production.

Breeding to improve phenotypes

The main component missing from algal strain development is a breeding programme. Such programmes have been essential to the manipulation of plant crops over the past century[31], and continue to be used for yield improvement and to protect crops. The breeding systems are well described for the model green alga *Chlamydomonas*, but not for others. The reason may be, in part, a lack of mating compatible isolates — as interfertile isolates have been identified only for *Chlamydomonas reinhardtii* laboratory strains[32] — or the strong push in most biofuel research towards molecular and transgenic technologies rather than breeding. Strains of algae could be improved by searching for phenotypic heterogeneity within interfertile wild-type isolates and the possibility of transgression (extreme progeny phenotypes beyond those of either parent). Their short sexual cycles and rapid growth allow their quick selection from an extremely large genotypic pool, creating the potential for breeding in microalgae to exceed in a few years the gains obtained from a century of breeding programmes in conventional plant crops.

Genetic engineering

Engineering of algal metabolism has an important role in the improvement of growth and biomass accumulation. With the use of next-generation sequencing technologies, our understanding of algal genomes has improved rapidly, especially the genes involved in metabolic responses[33–35]. Analysis of nitrogen deprivation in algae, which increases the accumulation of TAGs, has identified many responsive genes, including 86 annotated as transcription factors[35]. Insertional mutagenesis of one of these factors can influence TAG accumulation[36], suggesting the potential for metabolic engineering for lipid accumulation.

Whether alga mutants grow faster than their wild-type progenitor is unknown, but improving the growth rates of biofuel-producing microalgae certainly has potential. Researchers have not been able to achieve efficient homologous recombination in the nuclear genome of the commonly transformed laboratory algal strain *C. reinhardtii*, which has limited some research, but this is not the case for the marine alga and biofuel candidate *Nannochloropsis*[37]. Although there is no known gene in *Nannochloropsis* to target to improve growth, an example from the yeast deletion collection shows what might be possible: a single gene deletion resulted in a 7% increase in growth rate[38]; if this growth occurred in algae, such an increase would double the cells and biomass obtained over the time it takes a wild-type strain to divide 15 times (about the normal harvest cycle for algae). Further annotation of algal genomes will assist in identifying potential genes to target for manipulating the growth rate.

Cell density depends on the media used and algal species involved, and is typically highest for heterotrophic growth in bioreactors. The chlorophyte *C. vulgaris* has a particularly high cell density during heterotrophic growth, reaching 120 g l^{-1} (ref. 39). At high densities, the secretion of molecules that mediate cellular communication becomes important. Both *C. vulgaris* and *C. reinhardtii* have been shown to

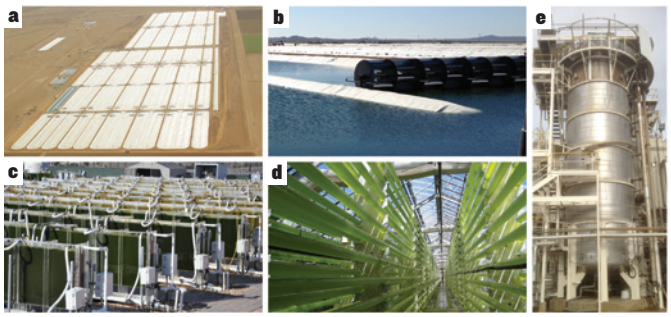

Figure 3 | Algae cultivation methods. **a**, Algal ponds of 0.5 ha and 1 ha are part of the first commercial-scale algal biofuel facility in the United States at Sapphire Energy's Integrated Algal BioRefinery. They cover an area 400 metres wide by 1,600 metres long at a location near Columbus, New Mexico. **b**, A single 1-million-litre paddle-wheel driven pond from the Columbus facility. **c**, A pilot-scale flat panel photobioreactor developed at the Laboratory for Algae Research and Biotechnology at Arizona State University in Mesa (image courtesy of Q. Hu). **d**, A commercial-scale tubular photobioreactor designed and constructed by IGV and operated by Salata in Germany (image courtesy of C. Grewe). **e**, An industrial-scale fermentation tank for heterotrophic cultivation of microalgae at Martek Biosciences, part of DSM in Heerlen, the Netherlands (image courtesy of D. Dong).

produce molecules that mimic quorum-sensing systems to inhibit bacterial growth[40,41]. Many other processes used to signal to other microalgal cells of the same species have also been identified, including autoinduction of cell division[42], premature cell death[43], allelopathy (production and release of metabolites that negatively affect the survival of another species[44]), and predation deterrence and warning systems for environmental stress[45,46]. Understanding cellular communication in algae might identify chemically regulated genes and allow the manipulation of pathways so that algal growth can exceed the current cell-density limits. For example, in *Escherichia coli*, a mutation in the *sdiA* gene, which is involved in the detection of *N*-acyl homoserine for quorum-sensing regulation, results in a 16% increase in final cell density compared with wild-type *E. coli*[47]. And in fungi, the molecule tyrosol can improve growth by selectively shortening the lag phase in a low-density culture without changing exponential growth[48].

Photosynthetic efficiency

The cultivation of algae in industrial photobioreactors or agricultural ponds aims to harvest as much solar energy as possible (Fig. 3). Efforts to improve photosynthetic efficiency have not been specific to algae; as a strategy, it has been proposed for increasing the yield of land plants to keep pace with increasing food demand where usable crop land is limited[49].

The genomes of most chloroplasts are capable of high rates of homologous recombination, and targeting specific genes in these organelles is not difficult in most species. Ideally, the photosynthetic characteristics of algae would be optimized for different photoperiods and light intensities. This optimization could be achieved by screening large numbers of mutants or by using the chloroplast photosynthetic machinery from species already adapted to these conditions. For instance, two strains of the green alga *Ostreococcus* with nearly identical morphology either grow near the surface (high-light strains) or at great depths (low-light strains), the antenna size in photosystems of low-light species is larger and, more strikingly, the electron flow is altered under low-light conditions[50]. Optimizing antenna size is also being considered in production systems as a potential means to limit the effect of shading and improve overall photosynthetic efficiency[51,52].

Manipulating the primary carbon-fixing enzyme Rubisco could also increase efficiency. Rubisco is the most abundant enzyme in all photosynthetic organisms, but also one of the least efficient. Hybrid enzymes from plant and algal species have already been shown to function in a different way from native enzymes[53]. In cyanobacteria, overexpression of Rubisco increased the photosynthetic rates (which is also potentially useful for the non-biomass derived biofuels in Box 1) when bicarbonate was used as a carbon source[54]. But Rubisco is not always rate limiting, its reduction (up to 50%) in plants and green algae did not have a negative effect on growth[55,56].

Photosystems within algae have evolved in response to their natural environments, and photosynthetic complexes are often remodelled in response to light, temperature or nutrient limitations[57]. Removing algae from their native environments protects them from occasional, but potentially lethal, environmental extremes. Engineering their chloroplasts to be optimal for the ideal environment, rather than the naturally occurring one, may improve photosynthetic efficiencies. A synthetic-biology approach has been used to introduce an exogenous chloroplast genome of *C. reinhardtii* into yeast cells[58]. A synthetic genome in yeast allows complex and rapid sequence manipulation to create synthetic chloroplast genomes; in fact, several photosystem proteins from *Scenedesmus obliquus* were found to function in *C. reinhardtii*[58]. This system could allow the creation of minimal plastids that are readily optimized for photosynthetic function.

Broadening lipid use

Lipid-productivity prospecting studies tend to examine neutral lipids (TAGs used for fatty-acid methyl esters). These constitute only a fraction of the total lipid content of an algal cell, which also includes polar lipids, such as phospholipids, sulpholipids and glycolipids. Using both types of lipids for fuel production would significantly increase the total yield of biodiesel that can be obtained from biomass. Academic and commercial enterprises have realized the importance of such research on this topic, and a direct transesterification method for total microalgal lipid content has produced appreciable levels of biodiesel even when there are undetectable levels of neutral lipids[59,60].

Genetic engineering could eventually enable algae to accumulate high levels of TAGs even under rapid growth conditions[61]. But being able to use essential polar lipids, as well as neutral ones, would ensure that a usable lipid source is available, irrespective of growth conditions.

Lipid and fuel quality

Lipid quality dictates many of the properties of the resultant fuel, including cetane number (a measurement of combustion quality in compression engines), oxidative stability, viscosity and cold flow[62]. Most common sources of biodiesel consist of unsaturated C_{16} and C_{18} fatty-acid methyl esters. Algae typically produce lipids between C_{14} and C_{20} in length. Unsaturated lipids usually yield fuels with a poor cetane number and low oxidative stability, whereas saturated fatty acids produce fuels at a higher cetane number and with greater stability but at the expense of their ability to be used at cold temperatures. Chemical strategies to improve the properties of biodiesel, such as reacting fatty-acid esters with alcohols other than methanol (ethyl and isopropyl esters have improved low-temperature properties), hydrogenation of lipids to increase saturation, traditional catalytic cracking and using additives to augment biofuel properties, can be used[62].

Attempts to increase lipid quantity through constitutive expression of transgenes in algae have had limited success owing to only minor expression of the transgene[63]. This is in contrast to overexpression in tobacco of the *Arabidopsis* genes *DGAT* (involved in TAG biosynthesis) and *LEC2* (a seed maturation and oil-storage regulatory gene), which increased the extracted fatty-acid yield[64]. However, lipid production is complex and may differ between some algae and higher plants, for example DGAT has been overexpressed in *C. reinhardtii* with no observable effect on lipid accumulation[65].

Another class of hydrocarbons found in plants and algae, the isoprenoids, are complex organic compounds that are ideal fuel precursors and could enhance the fungibility of biofuels[66]. Many isoprenoids are produced in the chloroplast, and a better understanding of their regulation and how best to modify the biosynthetic pathways might allow more biofuel precursors to be produced[67]. However, increasing lipid content and altering its type could have undesirable side effects: the green microalga

Botryococcus accumulates nearly 40% of its biomass in the form of the terpenoid hydrocarbon botryococcene[68] but at the expense of growth rate[69].

Nutrient use and recycling

Photosynthesis eliminates the need for costly organic carbon sources. However, media that can support a rich algal growth still require nutrients in the form of nitrogen, phosphorus and potassium, which are expensive[70] and — in the case of phosphorus — limited[71]. The cost can be managed by locating algal ponds or bioreactors near nutrient-rich wastewater streams, or by using feed sources such as anaerobic digester waste effluents[72]. Another option is to recycle the nutrients that remain in the algal waste after lipid extraction, rather than producing pellets to sell as a coproduct for animal feed. Evidence for the anaerobic digestion of algal waste[73,74] suggests that the next logical step is to optimize algae to grow on these waste streams. This strategy creates a process waste stream and provides nutrients, while using the energy stored within the waste algal biomass could help to run the production process. Chemical and engineering hurdles need to be overcome before this process will be feasible, and algae must be engineered to process these nutrients, including complex nitrogen and phosphorus, from the recycled algal waste. Other approaches for recycling these nutrients include supercritical water extraction to remove nearly all fatty acids but leave free ammonia and phosphates in an aqueous phase, which can then be used to supplement fresh growth media[75,76].

Glycerol recovery is also possible. This is one of the main by-products of the transesterification process that can serve as a carbon feedstock for heterotrophic algal growth. Grown on crude glycerol, *Chlorella protothecoides* has delivered biomass and lipid yields similar to growth on glucose[77]. Although the energy available in waste glycerol as the sole carbon source for energy production would not be viable, using this waste stream in photobioreactors as a feedstock supplement could certainly increase biomass yields.

Protection from pathogens

Crop protection is essential for outdoor pond systems and closed bioreactors. However, relatively little is known about the threats to algal farming even from known pathogens and grazing pests, and others may emerge with more intense farming. Long-term pilot outdoor facilities will be essential to determine the pathogen load in various environments. Pathogens of algae are extraordinarily diverse and include viruses, bacteria, fungi and other eukaryotes[78]. Apart from a virus infection of *Chlorella* discovered in the early 1980s (ref. 79), very few algal viruses have been studied. Grazing by zooplankton is also a pressure, but parasites are the greater threat to algal blooms in natural environments[80].

Strain selection can also greatly affect crop protection. Marine algae, such as *Dunaliella*, grow in halophilic media that few organisms can survive. *Nannochloropsis* species tolerate alkaline pH fluctuations[81], which may not be tolerated by many pathogens. Alkaliphilic cyanobacteria are already produced in large outdoor ponds for nutraceutical production in cultures that are almost entirely free of contamination.

As for many traditional crop plants, it may be possible to identify or breed algae that are resistant to the pesticides and antibiotics used to control unwanted species. Genetic transformation of algae has been established in many species aside from the model organism *Chlamydomonas*, and many of the methods used should be transferable to biofuel strains. Along with the requisite resistance gene, a low-cost and high-efficacy pesticide or antibiotic would need to be identified for treatment of ponds and bioreactors. Another, very different strategy for fighting bacterial contamination exploits the quorum-sensing systems that inhibit bacterial growth, and is currently being explored in mass cultivation of microalgae[82].

Improved harvesting

Harvesting, especially dewatering algal biomass, is one of the main costs in algal biofuel production. Most open ponds use a semicontinuous harvest strategy that removes roughly half of the algae, alleviating the need for re-inoculation. The algae are harvested after each doubling, so growth of a single generation per day would equate to a single harvest per day[83]. Alternatively, continuous systems to keep algae at a constant density and batch or complete systems are reminiscent of agricultural crops, removing nearly all algae during a single harvest.

Harvesting typically involves separation of the algae by sedimentation or flocculation, followed by filtration or centrifugation to remove the unwanted water. Drying the algae is an enormously energy-intensive step, which is one of the constraints on commercial development. Solar drying is an option, but still problematic with the current technology, owing to the time and space required.

Optimizing algae to ease harvesting can include increasing cell size, which helps with crop protection strategies and aids filtration. Algal strains can also be engineered such that the addition of a polymer or a change in an environmental variable triggers flocculation[84,85]. Conceivably, proteins involved in flocculation could be introduced and manipulated to respond to a particular growth state, as demonstrated in industrial strains of yeast[85,86]. Extraction of lipids from wet algae is possible using supercritical water extraction of algae with a moisture content of around 80% (ref. 76). Efficient wet extraction could remove the need for extensive dewatering and drying.

Outlook

Diminishing sources of fossil fuels and concerns over their use have made the need for efficient and sustainable biofuel production strategies more urgent. Large-scale biofuel production exists, but there is unease about its affect on food production and changes to land use. Algal biofuels could alleviate these worries. Algae can be grown quickly on non-arable land to produce large quantities of lipids that can be readily converted to fungible fuels. Although biofuel production from algae is still too expensive to compete with fossil fuels, it is an exciting prospective sustainable biofuel source.

BOX 1

Platforms for production

The designs for cultivation of algae to produce biofuel in ponds, bioreactors and photobioreactors all have unique characteristics, and how they produce fuel varies. In the agricultural model, different strains have been tested with different water sources and pond designs but, in all large-scale facilities, lipids are first accumulated inside the alga cell, then extracted after harvest and converted to fuel. The industrial model usually uses the same approach, whether using heterotrophic growth in fermenters or photosynthetic growth in photobioreactors.

Photobioreactors are expensive to scale, so direct secretion of fuel molecules from alga cells has attracted interest as a way of reducing the cost of harvesting. Fuels can be lipids that are naturally secreted by some algae[68,69] or algae engineered to secrete lipids from direct photosynthetic conversion[54,87]. Photofermentative growth is not a new concept for algae, and genetic modification has been aimed at lipid secretion and hydrogen production[88]. As algae are capable of accumulating sugars and starches from photosynthesis, conversion of these carbohydrates into ethanol, and secretion of this primary fuel has also been pursued[89]. In many cases, the accumulation of fuel and fuel precursors in an oxygenated environment will promote the proliferation of contaminating organisms, making crop protection strategies paramount to prevent catabolism of the secreted products. Although early-development fuel-secretion technology may reduce the need to harvest algae, any dense cultures used will eventually die, so the need to remove biomass from the growth medium is not completely eliminated (unless water and nutrient recycling are ignored).

With concerted effort, improvements can be made to the agricultural and industrial cultivation of algae for biofuel production. Industrial cultivation produces the most algae per litre, but is very expensive. Agricultural cultivation could in principle achieve cost efficiencies closer to those needed to compete with petroleum-based fuels, and with a reduced overall environmental impact, but its viability at a sufficiently large scale is only just beginning to be tested. Efforts to increase productivity but reduce input and cost through process engineering and the use of transgenic methods, and classical breeding aimed at developing domesticated algal crop strains, will benefit both strategies. With time, and driven by the continuing rise in the price of fossil fuels, technology and algal phenotypes will be developed to allow algal biofuel to compete with the commodities that currently underpin modern life — fossil fuels. ■

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