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Biodiesel from algae: challenges and prospects

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Microalgae offer great potential for exploitation, including the production of biodiesel, but the process is still some way from being carbon neutral or commercially viable. Part of the problem is that there is little established background knowledge in the area. We should look both to achieve incremental steps and to increase our fundamental understanding of algae to identify potential paradigm shifts. In doing this, integration of biology and engineering will be essential. In this review we present an overview of a potential algal biofuel pipeline, and focus on recent work that tackles optimization of algal biomass production and the content of fuel molecules within the algal cell.

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Introduction

With the need to reduce carbon emissions, and the dwindling reserves of crude oil, liquid fuels derived from plant material – biofuels – are an attractive source of energy. Moreover, in comparison with other forms of renewable energy such as wind, tidal, and solar, liquid biofuels allow solar energy to be stored, and also to be used directly in existing engines and transport infrastructure. Currently, bio-ethanol from, for example, corn starch, sugar cane or sugar beet, and biodiesel from oil crops such as palm and oilseed rape, are the most widely available forms of biofuel. However, there are two major issues over the sustainability of these first generation biofuels [1••,2]. Firstly, to provide a significant proportion of transport fuel, the growth of these crops would compete for arable land with food crops. In 2008, the UK used an estimated 47 billion liters of transport fuel, 53% of which was diesel [3]. If this were met using biodiesel from oilseed rape, it would require 17.5 Mha (Table 1), more than half the land area of the UK. Secondly, the overall savings in energy and greenhouse gas emissions over the lifecycle of the biofuel may be less than anticipated; for example for biodiesel from oilseed rape [2] and soya [1••] the input of energy required over the life-cycle is ~50% of the energy contained in the fuel.

Research into next-generation biofuels, such as ethanol from lignocellulose, offers the prospect of dealing with some of these concerns. In the past 2–3 years the production of biodiesel from algae has been an area of considerable interest [4,5••]. This is because: (1) algae have higher productivities than land plants, with some species having doubling times of a few hours; (2) some species can accumulate very large amounts of triacylglycerides (TAGs), the major feedstock for biodiesel production (Box 1); and (3) high quality agricultural land is not required to grow the biomass. However, several challenges need to be tackled to allow commercial production of diesel from algae at a scale sufficient to make a significant contribution to our transport energy needs. In this review, we consider recent strategies devised to tackle some of these challenges, in particular the optimization of algal biomass production and TAG content.

Algal biofuel pipeline

Figure 1 shows a strategy for the production of algal biodiesel. At each stage, there are many factors to be considered and optimized, including energy and material inputs (e.g. nutrients, and energy for mixing during growth), and appropriate treatment of waste products, such as spent media and residual biomass. The major features in the pipeline are as follows.

Choice of algal strain

A key consideration is the choice of algal strain. Algae are simple aquatic organisms that photosynthesize, but there are an estimated 300 000 species, whose diversity is much greater than that of land plants, shown in Box 1. There are many screening programs around the world surveying algal species in different locations for suitable strains, very often building on the pioneering studies in the Aquatic Species Program during the 1980s and 1990s [6•]. Complementing this, much current research work is focused on a small number of fast-growing microalgal

Table 1

Estimation of oil productivity from different crops. The estimates for algal growth are based on laboratory experiments [26] or pilot scale trials [12•]; using current technology, and on a large scale, the maximum productivity is unlikely to exceed 40 t/ha/y of oil [12•], resulting in ~45 000 L/ha/y of biodiesel. Current UK diesel use is 25 000 ML/y [3] equivalent on an energy basis to 27 000 ML/y of biodiesel. Thus with the productivity reported below, 17.5 Mha of land would be required to supply this using rapeseed (actual productivity), and 0.6 Mha with algae (assumed productivity[§]). For reference the total land area of the UK is 24 MHa.

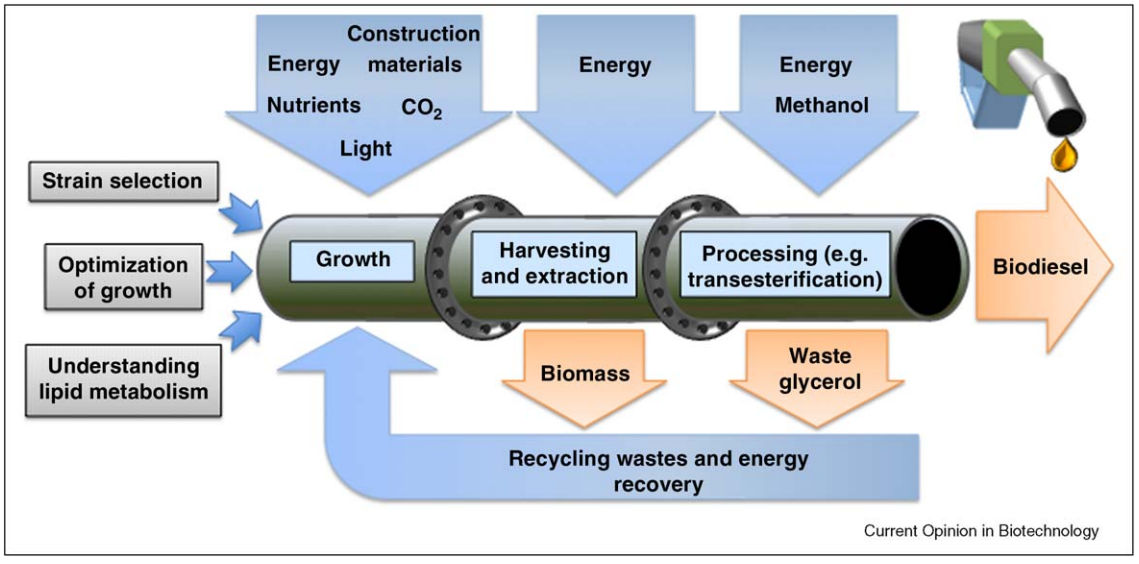
Crop	Oil content per tonne of biomass (wt% dry mass)	Oil production (t/ha/y)	Biodiesel yield (L/ha/y)
Oilseed rape (UK) [2]	40–44% (of seed)	1.4	1560
Soya [1••]	20% (of seed)	0.48	544
Jatropha [45]	30% (of seed)	2.4	2700
Chlorella vulgaris [26]	Up to 46%	7.2[§]	8200
Nannochloropsis [12•]	Up to 50%	20–30[§]	23 000–34 000

species which have been found to accumulate substantial quantities of lipids, albeit under specific conditions, as discussed below. Within the green algae, typical species include *Chlamydomonas reinhardtii*, *Dunaliella salina*, and various *Chlorella* species, as well as *Botryococcus braunii*, which although slow growing can contain over 60 wt% lipid (see Box 1), much of which is secreted into the cell wall [7]. Other important algal groups include the diatoms *Phaeodactylum tricornutum* and *Thalassiosira pseudonana*, and other heterokonts including *Nannochloropsis* and *Isochrysis* spp.

Growth of algal biomass and production of fuel molecules

In considering how to develop the algal biodiesel pipeline, knowledge can be gained from the current commercial growth of microalgae for high-value products, particularly when conducted on a large scale (eg species of *Chlorella*, *Haematococcus*, and *Dunaliella*). There are well-established harvesting and processing methodologies for the products, which can be produced economically, although essentially without regard for the energy inputs. Unfortunately, as a product of relatively low value to be produced on a very large scale, a different approach is necessary for algal-based biodiesel, and the major challenge is to ensure that it is not made at the expense of more energy than is obtained in the final fuel product. For the growth of algae for biofuel, particular concerns are: (1) whether closed or open bioreactors are feasible, (2) the strategies to be taken to avoid contamination by adventitious organisms, and (3) how nutrients and CO_2 should be supplied to the culture. For most microalgae, synthesis of fuel molecules such as TAGs is at the expense of growth, so conditions must be manipulated to optimize TAG production. These issues are discussed further below.

Figure 1



Algal biofuel pipeline, showing the major stages in the process, together with the inputs and outputs that must be taken into consideration by life-cycle analysis.

Box 1 Explanation of terms

Algae
Algae are photosynthetic aquatic organisms. The term is often used to refer specifically to eukaryotic organisms, thus excluding photosynthetic bacteria (such as cyanobacteria, which are also referred to as 'blue-green algae'). This review is restricted to eukaryotic microalgae. They may be unicellular (microalgae) or multicellular (macroalgae). The latter category includes seaweeds. The algae are very diverse in evolutionary terms. The red algae and green algae belong to one group, while the others, including diatoms, brown algae, heterokonts, and dinoflagellates are evolutionarily distinct. Some aquatic photosynthetic organisms, such as sea-grasses, are more closely related to flowering plants.

Antenna
This is a complex of chlorophyll and other pigments, such as carotenoids, and proteins that is used by plants and algae to trap light energy for photosynthesis. A significant part of the light energy may be lost; the remainder is used productively by the photosynthetic reaction centers to power photosynthesis.

Biodiesel
Biodiesel is a diesel fuel derived from plant or animal oils, usually composed of methyl esters of long-chain fatty acids. The detailed chemical composition, in particular the chain length of the fatty acids, depends on the source of the oil. Biodiesel is usually produced from the oil by chemical transesterification, where the glycerol to which long-chain fatty acids are esterified in the source oil is replaced by another alcohol. This is usually, but not exclusively, methanol. Biodiesel can be used in standard diesel engines, but is often blended with conventional diesel.

Fatty acid methyl esters (FAMEs)
These are chemicals in which long-chain fatty acids are esterified to methanol. Thus biodiesels are commonly FAMEs.

Life-cycle analysis
Life-cycle analysis (LCA) is often referred to as a 'cradle to grave' analysis. It attempts to quantify the environmental impact of all the processes that contribute to the provision of goods or services, in accordance with the ISO14040 standard. The two impact categories of greatest relevance to biofuels are the energy depletion potential, that is, how much primary fossil fuel energy is used over the lifecycle, and the global warming potential, equivalent to the total amount of greenhouse gas (mainly but not exclusively CO_2) released, measured in an equivalent mass of CO_2. So for example, in addition to the actual energy inputs in the growth of the biomass, the analysis would include all the energy used to make the nutrients and photobioreactor materials (sometimes referred to as embodied energy), up to and including the energy used to extract the raw materials from the earth (e.g. iron ore, coal, gas, oil, and uranium).

Photoinhibition
Photoinhibition is damage caused to the photosynthetic apparatus by excessive light levels.

Productivity
For crops – both land crops and algae – using solar energy for growth, productivity is expressed as a function of area. Thus lipid productivity is the rate of production of lipid per unit area per unit time (e.g. g/m^2/d or t/ha/y), and is equal to the rate of biomass production $\times$ lipid content of the biomass (wt%).

Harvesting and extraction

The biomass must be harvested, and processed to release the products such as TAGs, which can then be transesterified to produce biodiesel (as shown in Figure 2). The difficulty is in releasing the lipids from their intracellular location in the most energy-efficient and economical way possible, avoiding the use of large amounts of solvent, such as hexane, and utilising as much of the carbon in the biomass as liquid biofuel as possible, potentially with the recovery of minor high-value products. A key requirement is that the oil be released and extracted without significant contamination by other cellular components, such as DNA or chlorophyll. There is much scope for approaches based on selective decomposition of the cell wall, possibly using enzymes, and novel approaches minimising the use of solvents.

Final processing and use of co-products

The conversion of the extracted TAG into biodiesel uses transesterification with methanol to yield the methyl esters of the fatty acids present. This is now standard industrial technology, and little needs to be added. There is some evidence that the fatty acid composition of TAG expressed in some species would be higher in unsaturated acids than is specified in standards governing the allowable composition of biofuels. Use of the resulting glycerol makes an important contribution to the economics.

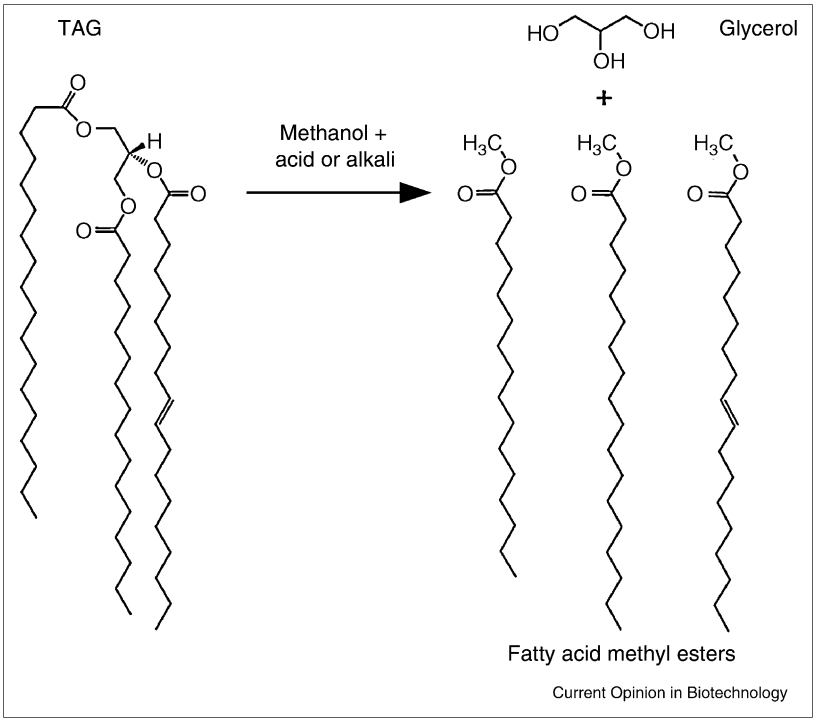
Overall energy balance for algal biodiesel production

Life-cycle analysis (LCA; Box 1) is an essential element in designing an algal biofuel pipeline, since it quantifies, systematically, the environmental burdens at every stage of production, from growth of the biomass through to final use of the fuel. Of particular importance are the usages of fossil fuel in production and the concomitant releases of fossil-derived CO_2. Energy inputs, such as the embodied energy in materials of plant construction and nutrients used, the electricity supplied from the national grid needed for mixing and pumping and any natural gas used for drying must be minimized. Furthermore, energy must be recovered from the waste products, and waste materials must be recycled to the process wherever feasible.

Closed photobioreactors or open ponds?

A major decision to be made is whether to use closed photobioreactors or open ponds (reviewed in Refs. [8–10]). Essentially, because light does not penetrate more than a few centimeters into a dense culture of algal cells, scale-up is largely on the basis of surface area, rather than volume as is the case with heterotrophic fermentations. Open ponds of large area are relatively cheap to build, and easy to operate, but there is the impossibility of controlling contamination, the difficulty of maintaining a constant environment for the culture, particularly its temperature, and the low cell density that can be achieved, arising from shading effects. The latter point

Figure 2



Esterification of triacylglycerides extracted from algal oil bodies for fatty acid methyl ester (biodiesel) production.

results in the need for extensive areas of land for the raceways and substantial costs for harvesting. To avoid microbial contamination, highly selective conditions have been used in some cases to guarantee dominance by the selected strain (e.g. *D. salina* in highly saline media, *Spirulina platensis* at high pH), but such conditions are not available for all species. Because of the drawbacks of open culture systems, much attention has been paid to closed photobioreactors, particularly with regard to the biomass productivity obtainable. Typical configurations tested at either laboratory or pilot scale have included vertical, flat plate reactors [9], annular reactors [11•], or arrangements of plastic bags operated as batches [12•], and various forms of tubular reactor, either pumped mechanically [13] or by air-lift [14]. Controversy surrounds the cost of scale-up, however, with estimates of capital and production costs varying widely. Contamination can be avoided in closed photobioreactors, but only if operated in a sterile – or at least hygienic – manner, which then adds to the expense.

In terms of energy, closed photobioreactors typically require energy for mixing (e.g. pumping, or energy used to compress gas for sparging), and have much embodied energy in the materials of construction, although this might be offset by the higher productivity of closed systems. Lardon [15••] performed an LCA for algal biodiesel production, and did not consider closed photobioreactors since they required too high an energy input. By contrast, Chisti [10] favored air-lift, tubular photobioreactors, and pointed out that while the productivity per unit area may only be slightly larger than a pond, the algae can be grown at a higher density, which reduces energy inputs down stream. Flat plate-air-lift reactors have been proposed on the grounds that the energy input for mixing is lower (~33% of the final energy contained in the biomass) than for an equivalent tubular system [9], in which the velocity has to be relatively high to promote turbulence [9,10]. One low-cost, and scalable, variant is the bag system [12•], but even this is unable to achieve a net energy ratio significantly above unity (i.e. energy contained in the biomass divided by the energy input), despite its low cost.

In addition to energy, the algal cultures must be supplied with nutrients and, in order to maximize the rate of growth, with CO_2 to support the high rates of CO_2 fixation in dense cultures. CO_2 may be available as flue gases from neighboring power or chemical plants, but on a large scale the distribution of CO_2 is problematic. Firstly, the energy costs of fans used for sparging extensive arrays of photobioreactors or raceways with flue gas could be significant. The flue gas, of course, needs to be free of growth inhibitors or toxic chemicals, which may require some pre-treatment. Lastly, the health and safety aspects of sparging large areas at ground level with a gas low in

oxygen (and potentially containing pollutants such as SOx and NOx) need to be carefully considered. On the other hand, the CO_2 capture may contribute to a favorable LCA. Additional benefits may accrue from the remediation of waste water if it is used to supply nutrients, particularly nitrogen (N). This is because the embodied energy of any nitrogenous fertilizer used significantly affects the energy balance [15••,16].

Downstream processing

In addition to the growth of the algae, the impacts of downstream processing on the energy balance must be considered. The life-cycle analysis of Lardon [15••] on biodiesel produced from *Chlorella vulgaris* grown in raceway ponds with either sufficient or limited N, suggested that the extraction of the oil from the algae with solvent resulted in a significant energy penalty, especially if the biomass were first dried before extraction. It was found that 1 MJ of energy in the biodiesel required an input of 1.66 MJ of energy, with a further 1.23 MJ potentially being recovered from the algal waste. A suggested approach for dealing with the significant amount of waste biomass, which contains energy and the majority of the nitrogen contained in the cells, is to use anaerobic digestion to convert the wet biomass to methane and a liquid fertilizer [17]. Recycling of spent biomass and nutrients from the downstream processing to the algal growth facility may help to reduce both expense and the energy costs of supplying nitrogenous fertilizer.

As noted earlier, using the glycerol resulting from transesterification could make an important contribution to the economics and LCA. Various workers have shown that species of green algae and diatoms will grow mixotrophically on glycerol [18,19], so that there is scope to feed it back to the fermentation. However, trials with the impure glycerol arising from transesterification are needed, rather than with purified glycerol, and at scale, since all studies to date are laboratory-based.

Thus, with current methodologies, the viability of algal biodiesel is only marginal. It might even be better to burn biomass directly in an existing power station to substitute for coal, rather than to extract the lipids [16,20]. It is therefore unlikely that engineering approaches alone will provide the means to allow full commercialization of algal biodiesel production. Instead, there must be an integration of the engineering with discoveries in algal biology. Two areas in which there is considerable research activity at present are (1) optimising algal growth systems and (2) maximising the rate of production of TAGs. Increasing the growth rates and concentrations of cells in culture will help to offset the embodied energy of materials used in the photobioreactors or ponds, and reduce the cost of downstream processing. Increasing the TAG content per unit mass of cells reduces the amounts of nutrients needed, and also minimizes the amount of residual biomass, which may contain a significant proportion of the total energy, is minimized.

Optimization of algal growth – the importance of light

Maximal production of algal biomass is essential to ensure the best possible outcome for the energy balance calculations. Although the biomass yield of many algal species is very high, it is important to note that this is constrained by the laws of thermodynamics [21]. Eight photons of photosynthetically active radiation (PAR) (~48% of the incident solar flux) are required to fix one molecule of CO_2 into carbohydrate, resulting in a maximum photosynthetic efficiency (not including respiration) of about 12% [22,23]. When respiration is taken into account, the maximum efficiency falls to ~9% [9]. Over short durations and in favorable conditions (low to moderate light levels) reported efficiencies of ~4.5–7% are typical in both ponds and photobioreactors [11,13], which, in a pond, translates to a yield of 30–40 g of dry biomass per square meter of pond per day.

Manipulating the delivery of light

Irrespective of the method used to grow the algal biomass, an important consideration is the need for optimal light delivery to all of the cells within the culture. The photosynthetic apparatus consists of photosystems I and II (PSI and PSII), where light energy is used for photochemical reactions, surrounded by antenna complexes that harvest the light energy and pass it on to the photosystems. Algae have evolved to absorb more light than is needed for their own photosynthetic requirements, at the expense of competitors; the excess light energy is dissipated as heat and fluorescence. While this confers evolutionary advantage, it results in a significant reduction in the amount of PAR that can penetrate dense cultures, so that optimal depths are only one to a few centimeters (e.g. [11•]). This has been estimated to cause a reduction in dry weight yield of three-fold or more [24]. Moreover, at high light levels not only is there less efficient use of absorbed light energy, but also biochemical damage to the photosynthetic machinery can occur (photoinhibition), making light energy utilization even less efficient. Thus the highest photosynthetic efficiencies are realized at low light intensities.

Photobioreactors, in contrast to ponds, offer the opportunity to optimize the light path, the extent to which the incoming light is diluted, and also the frequency of the light–dark cycle seen by an algal cell as it travels from deep in the culture to the illuminated surface. For example, using arrays of vertical annular columns, the productivity was significantly greater than using a horizontal pond [11•]; both received similar amounts of light per unit land area, but in the former case the light was spread over a larger reactor area. Larger surface areas however imply more embodied energy used in the

materials of construction. Rapid mixing can equalize the time spent by cells near the surface, where they are exposed to very high light levels, but at the cost of energy supplied to drive the mixing.

Manipulating the capture of light

A completely different strategy to optimize light utilization is to reduce the antenna size within the algal cells [24••]. This can be achieved by growth at high light intensity, but the change is readily reversible, so that if cells are subsequently transferred to low light, the antenna size increases again over the course of a few hours [25]. Furthermore, the cells grown at high light levels may show significant levels of photoinhibition. Manipulation of antenna size through nutrient levels has received less attention. However, conditions of nutrient deprivation leading to increased productivity of TAGs (see next section) may also cause a reduction in chlorophyll levels per cell [26], suggesting that nutrient deprivation may have multiple beneficial consequences for feedstock production.

Reduction of antenna size has also been achieved by mutation of genes responsible either for components of the antenna, or for regulation of antenna biogenesis. For example, Polle *et al.* [27] demonstrated a reduction of more than three-fold in the amount of PSII antenna chlorophyll in a strain of *C. reinhardtii* lacking chlorophyll *b* as a result of a mutation affecting the chlorophyll *a* oxygenase required for chlorophyll *b* synthesis. Perhaps surprisingly, there was little effect on PSI. Similarly, a *C. reinhardtii* strain lacking a number of carotenoid classes also showed a significant reduction of PSII antenna size but little effect on PSI [28]. Tetali *et al.* [29] found that reduction in expression of the *Tla1* (truncated light-harvesting antenna) gene of *C. reinhardtii* led to a reduction in the PSII antenna size by about one half, and a reduction in the PSI antenna size by about one third. Wobbe *et al.* [30•] demonstrated a reduced PSII antenna in *C. reinhardtii* following mutation of cysteine residues in NAB1, a redox-regulated cytosolic protein that represses translation of the PSII antenna protein LHCII mRNA. In all these cases, the modified strains exhibited enhanced photosynthetic efficiency at high light levels. Although these are promising results, there is the possibility that reduced antenna size could result in reduced fitness, thus compromising the overall productivity of the strains. Although this may not be a problem if strains are grown in photobioreactors where contamination can be controlled, it may lead to significant reductions in overall productivity if strains are grown in outdoor ponds or raceways where competition from adventitious organisms is likely.

Finally, enhanced efficiency of light utilization could result from the use of mixed cultures able to use different wavelengths of incident light. For example, the cyanobacterium *Acaryochloris marina*, which contains chlorophyll *d*, can utilize light with longer wavelengths than chlorophyll *a*-based organisms [31], allowing it to live in environments where wavelengths compatible with chlorophyll *a*-based reaction centers have already been absorbed. In principle, mixed cultures including *A. marina* might therefore be able to utilize the small amount of light energy at longer wavelengths that would otherwise be unused.

Maximizing the TAG content in algae

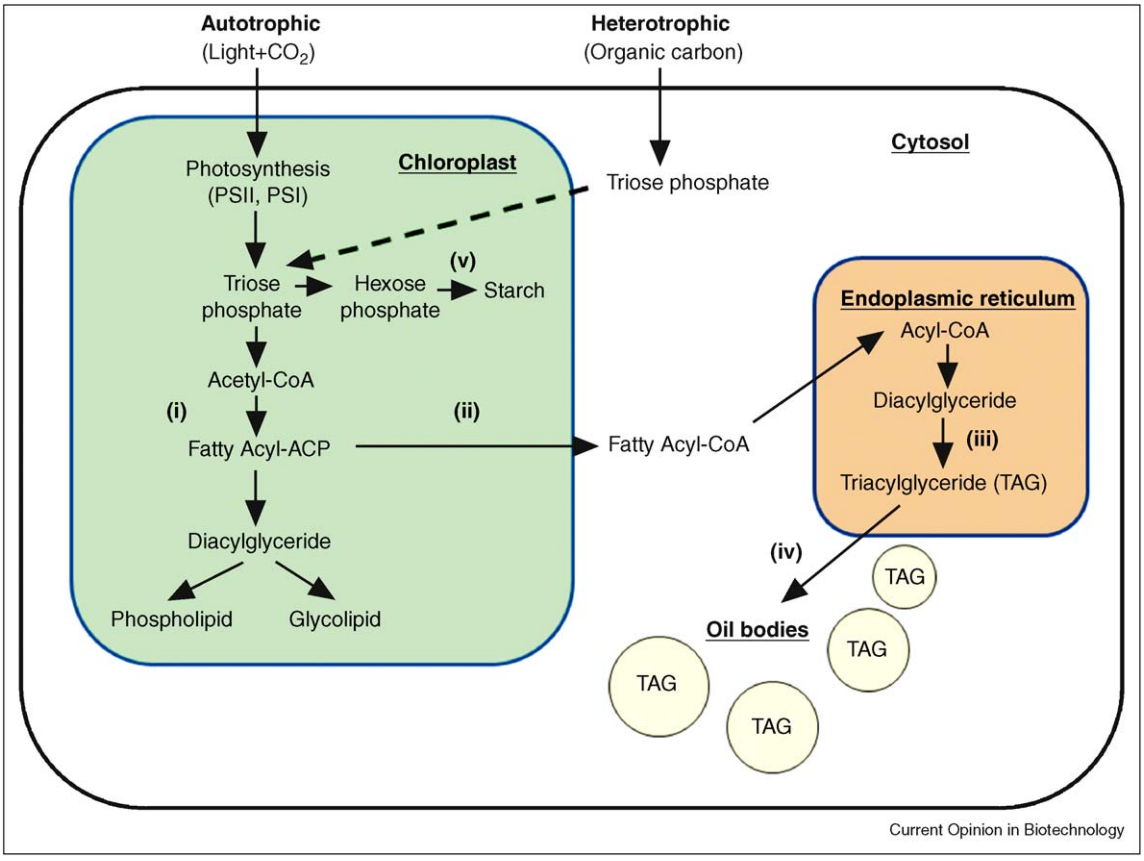
The yield of biodiesel from algae depends not just on the concentration of biomass achieved, but also on the oil content of the individual cells. Figure 3 provides a schematic of the biochemical pathways concerned. In general, productivity and lipid content are inversely correlated, and stress conditions such as deprivation of N or phosphate, which limit cell growth, also increase lipid content [12•,32]. For example, while the lipid content of *C. vulgaris* grown under nutrient-sufficient conditions is between 14% and 30% of dry weight [33], values of up to 70% of dry biomass have been reported under nutrient deficiency [12•]. The underlying principle is that where there is insufficient N for protein production necessary for growth, excess carbon from photosynthesis is channeled into storage molecules, such as TAGs or starch (Figure 3), and protein content may be reduced.

Manipulation of nutrient supply

Several recent studies have described growth conditions that can be used for the industrial scale-up of lipid production from algae. The two-stage process suggested by Rodolfi *et al.* [12•] achieved 0.2 kg/m^3/d oil for photosynthetic microalgae. In this set-up, cells were first grown under nutrient-sufficient conditions for biomass accumulation followed by nutrient deprivation for lipid synthesis. However, a study by Stephenson *et al.* [26] found that the most effective strategy for achieving high lipid contents in *C. vulgaris* was to allow cells to deplete their N naturally rather than to transfer them to a medium completely lacking N.

Many algae can grow heterotrophically with an external carbon source, rather than photosynthetically (Figure 3). This alters the N:C ratio, and thus has the same effect as growing algae with reduced N levels under autotrophic conditions, namely elevated lipid production. For example, *Chlorella protothecoides* had 55 wt% lipid when grown heterotrophically, four times higher than under autotrophic conditions [34]. A further enhancement was seen when sugars were added to photosynthetically grown cells of *C. protothecoides*, resulting in lipid levels of up to 11.8 kg/m^3/d [35•]. Similar to the protocols described above algae were grown in two phases: at the end of the log-phase, algal cells grown autotrophically were transferred to a medium containing 45 g/l glucose.

Figure 3



Basic overview of the pathway of carbon capture and lipid biosynthesis. Only the major steps are indicated for clarity, for detailed pathways see Refs. [5••,39]. Precursor fatty acids are synthesized *de novo* in the chloroplast, using either carbon fixed during photosynthesis, or from an exogenous supply of organic carbon; the exact nature of what enters the chloroplast is unknown in algae (dashed line). Free fatty acids are exported from the chloroplast and then converted to TAGs in the endoplasmic reticulum (ER), where they bud off into oil bodies in the cytosol. Key: (i) = acetyl-CoA carboxylase (ACCase) and fatty acid synthase (FAS); (ii) = fatty acid thioesterases and acyl-CoA synthetases; (iii) = TAG biosynthesis enzymes, including acyl-CoA:diacylglycerol acyltransferase (DGAT); (iv) = oil body formation; and (v) = ADP-glucose pyrophosphorylase and starch synthase.

At the same time, N levels were reduced in order to induce lipid biosynthesis. Under these conditions, 58.4% oil content per dry weight was achieved, with no alteration in fatty acid composition [35•]. On the other hand, the source of sugars or other organic carbon is an issue, as is the potential for contamination of cultures by heterotrophic fungi and bacteria.

Metabolic engineering of lipid production pathways

With the advent of genome sequences and molecular tools for algae, there is the possibility that metabolic engineering may provide important and significant improvements for algal biodiesel production, for instance by increasing yields of TAGs, or engineering pathways for novel biofuel molecules. The latter approach has been employed widely in yeasts and *E. coli* (for reviews see Refs. [36,37]). For example introduction of a monoterpene synthase from sweet basil into *Saccharomyces cerevisiae* resulted a strain that not only synthesized large amounts of the monoterpene geraniol, but also secreted it into the medium, thus avoiding the need for extraction [38]. In higher plants, several studies have explored the effects of overexpression of enzymes of lipid synthesis on TAG production (reviewed in Ref. [39]). Little alteration in oil content was seen in plants with elevated levels of acetyl-CoA carboxylase, the rate limiting step in fatty acid biosynthesis (Figure 3, step (i)), possibly because of the complex regulation of this enzyme. By contrast, overexpression of thioesterases (step (ii)) [40] to encourage exit of fatty acids from the chloroplast to the cytosol/ER where TAGs are synthesized, and of enzymes of TAG production (step (iii)), such as acyl-CoA:diacylglycerol acyltransferase (DGAT, [41]), proved much more successful in increasing the yield of fuel molecules. These observations indicate the importance of understanding the metabolic pathways involved, and their underlying regulation, in particular the increased energy burden of any new pathway, and probably more importantly, the interdependence of pathways.

At present, we are far from understanding fully the detailed molecular biology and regulation of lipid body metabolism in algae, and although bioinformatics of sequenced algal genomes indicates that the same pathways are likely to operate, there has been little experimental verification of putative enzyme activities [5••]. Nonetheless, two recent papers have made some significant observations [42•,43••]. As described above, lipid accumulation can be induced by N deprivation. This inducible process provides a useful experimental basis for observing changes in gene transcript, protein and metabolic activities during lipid accumulation in algae. Moellering and Benning [42•] used N depletion experiments, in combination with RNAi suppression, to assess changes in the lipid and protein composition in *C. reinhardtii* during lipid droplet formation (Figure 3, step (iv)). In cultures transferred to N-depleted media, they were able to confirm that after 72 h the total fatty acid content on a per cell basis increased by 2.4-fold, of which 65% of the total fatty acids were esterified to TAG in oil bodies, when compared to the initial culture. This indicated that TAG formation is increased by *de novo* synthesis of fatty acids. Proteomic analysis identified a 'major lipid droplet protein' (MLDP), which was highly abundant in the lipid bodies. The *MLDP* mRNA transcript abundance also followed the corresponding increase in lipid droplets after N depletion. RNAi lines of *C. reinhardtii* with a 55–60% reduction of the MLDP transcript produced lipid droplets that had a diameter 40% larger than the control lines, implying that the function of this protein is to regulate lipid droplet size.

Another way of increasing lipid yield is to delete other 'redundant' pathways in the organism, so freeing precursor metabolites for the desired biofuel production. Wang *et al.* [43••] have successfully measured an increase in the abundance of TAG lipid droplets in the *C. reinhardtii* starchless mutant (*sta6*), deficient in ADP-glucose pyrophosphorylase (an essential enzyme in starch production; Figure 3, step (v)). After 48 h of N depletion, wild-type cells had increased their lipid droplet content by 15-fold, but there was a 30-fold increase in lipid droplets in the *sta6* starchless mutant algae. Ultimately, this resulted in a higher concentration of TAG per cell, as after 18 h of N starvation the *sta6* had on average 17 ng TAG (10^3 cells^{-1}) compared to 10 ng (10^3 cells^{-1}) in the wild-type cells.

Conclusion and future prospects

We are still some way from realising the undoubted potential offered by algal biodiesel. Life-cycle analyses suggest that – using current methodologies – the process is marginal in terms of positive energy balance and global warming potential. Prospective schemes for the scale-up of algal production need to be informed by careful process modeling and LCA from the design stage. Without careful assessment of the energy balances and environmental impacts, there is a danger that many proposed schemes would be nonsensical from the point of view of sustainability. Moreover the lack of data from real-life demonstrations means that economic assessments are essentially hypothetical, and there is a pressing need to conduct pilot studies at a realistic scale and under prevailing weather conditions, so as to assess productivities likely to be achieved in practice. Finally, selecting high lipid producing strains optimized to regional climate conditions and to the large-scale production of algae biomass, and preferably also amenable to metabolic engineering, will be crucially important.

Nevertheless, the opportunities for innovation and tangential approaches to tackling some of the challenges are considerable, in particular by integration of engineering and biology, since neither is likely to yield an appropriate solution in isolation. For example, a change in algal lipid composition under stress conditions is often observed, which means that the oil cannot be used directly for biodiesel production [12•,32]. However, it has been reported that the freshwater green microalga *Parietochloris incisa* enhances not only its TAG production under N starvation but also the proportion of arachidonic acid, a valuable nutraceutical, in TAGs [44]. In this case, the valuable by-product could make biodiesel production from this species viable. Thus establishing the feasibility of algal biofuel production regardless of energy input, will be an important step in providing the platform for optimization, and also for establishing promising lines for future research. This is essentially what has happened with first generation biofuel where, despite the concerns over food prices, land use and so on, it has led to the development of infrastructure, policies, and know-how.

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