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SUSTAINABLE AND RENEWABLE FUELS (X CAI, SECTION EDITOR)

Potential of Bioenergy Production from Microalgae

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Abstract There is increasing interest for sustainable bioenergy production to mitigate greenhouse gas emissions and reduce reliance on fossil fuels. Biofuel can be generated from a wide variety of feedstock types including algae. Algae are an attractive feedstock for the production of liquid and gaseous biofuels that do not need to directly compete with food production. However, both the sustainability and the economic viability of algal biofuels have been questioned, particularly with regard to high carbon and fertiliser input requirements, and high cultivation and production costs. Improved understanding and modifications at a biological level, of algal genetics, carbon storage metabolism, photosynthesis and algal physiology, have the potential for significant advances in algal biofuel feasibility. This is being driven by advances in genomic technologies to provide the potential for genetic and metabolic engineering, plus the development of high-throughput techniques for the screening of natural strains for suitable biofuel characteristics.

Keywords Algae biofuel · Biodiesel · *Chlamydomonas* · Forward genetics · Genetic manipulation · Hydrogen production · Life-cycle assessment · Lipid metabolism · Microalgae · Transcriptomics

Introduction

The use of fossil fuels as the primary source of energy is an unsustainable practice. Remaining oil reserve levels are unknown and the most optimistic estimations suggest oil reserves will last for a number of decades rather than centuries [1]. However, the environmental impacts of fossil fuel consumption have been widely demonstrated, with a significant issue being the rising levels of atmospheric greenhouse gases (GHG) contributing to global climate change [2]. Therefore, there is increasing interest for sustainable alternatives such as bioenergy production to mitigate GHG emissions and reduce reliance on fossil fuels. In particular, there is a need for alternative liquid transportation fuels and it is for this reason that there has been significant development and commercialisation of biofuels in recent years. Biofuel can be generated from a wide variety of feedstock types, including various plant sources such as plant oils, sugars, starch and lignocellulose biomass from plant waste or energy crops; from animal oils and biomass; from organic waste; and from cultivated microorganisms [3, 4]. However, many food sources of biofuel feedstock such as wheat grain, corn starch and sugarcane, currently used extensively for commercial biofuel production, have been criticised because of their competition with food crops for agricultural land, water and nutrients [5, 6]. The use of non-food 'energy crops' such as switchgrass and *Jatropha curcas* are being examined for biofuel production and may be promising for the future, but there may still be some issues of agricultural resource conflict [7]. In contrast, an alternative, potentially sustainable, biofuel feedstock source to be considered is algae. This review highlights some of the current challenges of biofuel generation, focussing mainly on microalgae, and will examine some of the recent research on biological understanding and genetic manipulation (GM) of microalgae, which may allow biofuel production from these organisms to be economically and sustainably viable in the future.

Biofuel Products from Algae

Algae are a diverse group of photosynthetic organisms with a range of unicellular to multicellular forms that are found in the ocean, freshwater bodies, on rock, soils and vegetation. They can be broadly divided into macroalgae, which include multicellular seaweeds, and microalgae, which are small

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unicellular algae, found in a wide variety of environments and comprising of many evolutionarily distinct organisms [8]. As autotrophic organisms, algae use solar energy and atmospheric CO_2 to synthesise organic macromolecules including lipids and carbohydrates that can be converted into biofuel. Thus, algae have the potential to provide a theoretically carbon-neutral fuel that requires minimal inputs. Some algae species can also be grown heterotrophically for higher productivity but then require carbon feed. A variety of scenarios for biofuel production from algae exist (Fig. 1). All types of algae can be used as a feedstock for the production of liquid and gaseous biofuels by thermochemical conversion methods such as pyrolysis, gasification and hydrothermal liquefaction [9]. Macroalgae accumulate very low quantities of oils, but are a good source of carbohydrate that could be converted into bioethanol or biomethane by biological conversion methods such as anaerobic digestion and fermentation. Microalgae are of particular interest because of the high yields of oil available in some strains, principally the glycerolipid triacylglycerol (TAG), that can be converted into biodiesel by transesterification [3, 10]. High oil productivity of microalgae suggests that substantially less land area would be needed than for oil crops such as soybean or oilseed rape to produce the same quantity of biofuel [11]. In addition, microalgae are of interest because of the ability of some strains to produce hydrogen that could be used as a fuel source [12•, 13]. Finally, some microalgae, in particular *Botryococcus braunii*, can produce high concentrations of unusual triterpenic hydrocarbons that can be readily converted into fuels by conventional hydrocracking and distillation procedures [14].

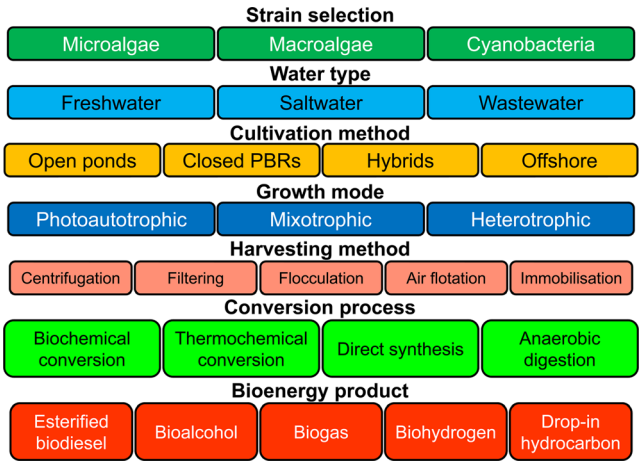

Fig. 1 The building blocks of algae biofuel production. The various scenarios for biofuel development from algae are represented. Many options are available with regard to algae type and strain choice, including both eukaryotic algae and prokaryotic cyanobacteria, the source of water for cultivation, cultivation method and mode of growth, the method of algae harvesting and the biofuel conversion process, which will partly determine whether a metabolite such as triacylglycerol is extracted from the algae, and will determine which type of biofuel will be generated

However, a challenge with this particular organism is its very slow growth and relatively inefficient oil extraction.

Overall, the significant attraction of algae as a biofuel feedstock is that it could be cultivated on non-agricultural land in open ponds, photobioreactors or in some cases under heterotrophic fermentative conditions. The cultivation could occur in conditions that do not need freshwater, as many strains can grow efficiently in saltwater or wastewater [10, 15]. Furthermore, algae productivity could be enhanced through the exploitation of waste CO_2 such as from flue gas sources [16]. These various cultivation and biofuel generation scenarios (Fig. 1) mean that estimations of commercial-scale algal biofuel production (algal crude oil) can vary widely, potentially from 8,000 to 140,000 L ha^{-1} y^{-1} [11, 17]. The commercial production of algae biofuel is still limited, particularly from autotrophic open pond cultivation, but appears to be expanding for heterotrophic cultivation. For example, the US-based company Solazyme produces algal oil from heterotrophic cultivation for biodiesel production, and produced over 500,000 L in 2010–2011, and plans to have production facilities for millions of litres of oil by 2015 [15, 18]. However, despite the significant research interest and private investment in algae biofuel generation over the last decade, both the sustainability and the economic viability of algal biofuels have been questioned.

Challenges for Sustainable and Economically Viable Algal Biofuel Production

Life-cycle assessment (LCA) has been used to predict the potential environmental benefits and implications of large-scale algal biofuel production. A wide variety of LCA studies have now been published, mostly for the analysis of biodiesel production from microalgae. However, the predictions and conclusions from these studies have been highly variable and unable to be directly compared, partly because of the wide variation in microalgal cultivation, harvesting and processing methods that can be modelled (Fig. 1), but also because of different LCA methods, hypotheses and parameters being used [19•, 20•, 21]. For example, with regard to modelled environmental impacts of biofuel production, a comparison of 15 LCA studies found that there was significant variation in GHG emission balance results as a result of different modelling methodologies and assumptions, with some showing a negative environmental impact while others are positive [20•]. Furthermore, while most of these studies assess GHG emission balance, few assess other impacts such as eutrophication or land use. One approach to overcome these comparison issues is to normalise LCA results. A recent analysis of six normalised LCA studies suggests that microalgae biodiesel production can provide a positive GHG emission balance and positive energy balance, but at values that

are equivalent to those of conventional crop-derived biodiesel [19•]. The overall consensus indicates that algal biofuels can be beneficial in terms of GHG emissions. For example, a recent analysis of a pilot-scale microalgae open-pond and hydrothermal liquefaction facility run by Sapphire Energy indicated that such a process would provide lower GHG emissions than petroleum fuels and corn ethanol but with a downside of significantly lower energy return on investment than petroleum fuels [22].

LCA studies have also predicted substantial negative environmental impacts, notably a high freshwater use and nutrient (fertiliser) requirement. For example, if large-scale algal biofuel production was implemented to produce 5 % of the current transportation fuel demand of the USA, the use of 1–2 million t of phosphorus (P), 6–15 million t of nitrogen (N) and 3.1–3650 L of freshwater per L biofuel has been estimated [23, 24]. The variation in water requirement varies depending on whether freshwater or marine algae are cultivated, indicating that freshwater cultivation is clearly unsustainable. However, such a fertiliser requirement is clearly untenable as it would directly compete with fertiliser requirements for food production, while the increasing scarcity of P fertiliser and the high GHG footprint of N fertiliser would be a further concern [25, 26]. The suggested use of wastewater to partly mitigate freshwater use may also allow recycling of waste P and N nutrients, which are abundant in many municipal wastewater sources. Furthermore, the cost benefit of using the algae for remediating wastewater may allow a reduction in biomass production costs [27–29]. Wastewater can be a challenging and toxic environment for algal cultivation and requires the development of strains that can grow successfully [30–32]. However, there is a limited supply of wastewater, and nutrients from this source will not be sufficient as an alternative to fertiliser [33, 34]. Therefore, wastewater cultivation of algae should be limited to remediation applications and any energy return used to reduce waste treatment costs.

The current cost of biofuel production from algae is hard to determine, particularly as there are no commercial-scale production facilities. A recent estimate of biodiesel cost has a range of US$0.42–0.97 L^{-1}, which was felt by the authors of the study to be close to commercial reality [35]. Despite a wide number of start-up companies and larger multi-national corporations investing in algal fuels over the last decade [34], there has been no evidence of a significant reduction in cost, with one report that it cost Solazyme approximately US$17 L^{-1} to produce biodiesel using heterotrophic cultivation methods [15]. However, the current low cost of petroleum fuels coupled with high projected costs of algal biofuel production based on existing technology gives a consensus that these fuels are non-viable commercially in the short term [34]. There are a variety of factors contributing to high production costs [34]. These include low biomass productivity particularly for open pond-based cultivation [36] and where CO_2 input is low or inconsistent [37], input costs including fertiliser and carbon if algae is grown heterotrophically [34], high cultivation infrastructure and maintenance costs particularly for photobioreactor-based cultivation, and high harvesting, product extraction and processing costs [38]. Clearly, there are many technological hurdles to overcome and it is therefore unsurprising that the current short- to medium-term focus with commercial algal biotechnology is for the production of high-value nutraceutical, pharmaceutical and cosmetic products such as beta-carotene and omega-3 fatty acids rather than biofuel [39].

Biological Solutions for Algal Biofuel Advances

While improvements are being made to cultivation technology, energy-efficient harvesting methods and oil extraction procedures, improved understanding and modifications at a biological level have the potential for significant advances in algal biofuel feasibility. It has been argued that a development of algae that is analogous to the breeding and domestication of crops is required to really exploit the potential of these organisms for both biofuel usage and other applications [40]. For biofuel development, this requires improved understanding of algal genetics, lipid metabolism, photosynthesis and algal physiology to attempt to improve algal growth and biomass productivity, improve light harvesting and CO_2 use efficiency for photosynthesis, improve oil productivity and extraction, improve nutrient use efficiency and improve cell autoflocculation for more efficient harvesting. Advances in genomics technologies and genetic transformation provide the potential to manipulate algae genetically [11, 40–43]. However, in addition to the use of genetic and metabolic engineering, it is essential to make use of the significant natural variation and diversity that exists within algal populations, and to consider non-GM methods.

Genomic Approaches for Gene Discovery

Potential improvements of biofuel characteristics of algae through genetic engineering depend on the identification of target genes. The continuing availability of sequenced algal genomes [41, 43] and recent advancements in transcriptomic sequencing technology, particularly with the model microalga *Chlamydomonas reinhardtii*, have increased the scope of our genetic understanding of microalgae [44–46], not only allowing the identification of key genes, but also for finding novel genetic regulators [47•], which could be targets for GM.

There is strong interest in understanding carbon metabolism and its regulation in context to lipid (specifically TAG) accumulation in microalgae [48, 49]. Our understanding of carbon storage metabolism pathways in chlorophyte microalgae, in particular *C. reinhardtii* has expanded greatly

in recent years through the use of comparative genomics and transcriptomics, and the reader is directed to recent reviews for a detailed description of lipid and starch metabolism in microalgae [40, 41, 50•]. The concentration of TAG accumulated by algal cells has been shown to significantly increase under environmental stress, with N starvation considered the best trigger for TAG accumulation, but the starvation of nutrients such as sulphur (S), P, zinc, iron and salt stress also promotes both TAG and starch biosynthesis in algae [51, 52, 53••, 54, 55]. While manipulation of nutrient deficiency in cultivation conditions can be used to enhance lipid productivity in mass cultures [56], the identification of these stresses has other benefits. Such consistent TAG and starch biosynthesis inducers such as N starvation are an excellent tool to understand the transcriptional and metabolic changes that occur within the cell during the onset of carbon storage. For example, this has been proved as a strong tool for the identification of some of key lipid metabolism enzymes [40, 44, 47•, 53••].

Starch-less mutants of *C. reinhardtii* (such as *sta6*) produce more lipid than wild-type strains because of an increased carbon pool [57]. A transcriptomic comparison of *sta6* and wild-type *C. reinhardtii* under N-starved conditions examined the effect of redirecting carbon from starch biosynthesis toward TAG accumulation, and found several transcriptional changes of key rate-limiting carbon metabolism enzymes, giving a hope of finding a key regulator for carbon flux in N-limited conditions [53••]. Despite *sta6* being unable to produce significant starch, various starch biosynthesis enzymes are up-regulated in response to N starvation during early stages of growth, suggesting that starch synthesis is a programmed response to N starvation regardless of output. When grown under N starvation and boosted with additional exogenous carbon in the form of acetate, many enzymes involved in acetate metabolism increased highly in *sta6*, along with an acyl-CoA:diacylglycerol acyltransferase (DGAT), a glycerol-3-phosphate dehydrogenase, both key enzymes in TAG synthesis, and two lipases [58•]. This coordinated increase in acetate metabolism in *sta6* suggests an up-regulated increase in acetate use and carbon flux for the generation of lipid storage in *sta6*, making this carbon flux pathway a potential regulator of TAG biosynthesis in N-starved conditions. Furthermore, the study highlights up-regulated enzymes, such as the DGAT, as interesting GM targets for enhanced storage metabolite synthesis.

Similar transcriptomic studies have been performed under various nutrient starvation conditions to indicate key genes in carbon storage metabolism [44, 45, 47•, 59•]. These include two DGATs, and a phospholipid diacylglycerol acyltransferase, another key TAG biosynthesis enzyme, plus a putative transcriptional regulator of N-induced lipid metabolism, called NRR1, which when mutated causes a reduction in N starvation-induced TAG accumulation [47•]. S starvation is also of interest as this stress can not only induce the accumulation of TAG and starch, and correlated up-regulation of specific lipid metabolism genes [45], but can also induce the production of hydrogen [13]. Transcriptomic analysis of *C. reinhardtii* undergoing hydrogen production following S starvation shows substantial remodelling of primary metabolism, but also the identification of potential regulatory genes that could be future targets for manipulation to increase hydrogen production in this microalgae [59•].

Targeted Metabolic Engineering

Advances in our understanding of microalgal lipid metabolism, and of the genes encoding these pathways, such as from transcriptomic analyses, provides the possibility of developing economically viable strains of microalgae for biodiesel through targeted metabolic engineering (Table 1). Manipulation of microalgae requires the ability to stably transform the organism with a transgene, which until recently has been challenging for all but a select few species [41], and even though nuclear genome transformation is efficient in a species such as *C. reinhardtii*, stable expression can be variable [60] and targeted nuclear genome manipulation has not been demonstrated. However, of particular interest was the recent observation that nuclear genome insertion in *Nannochloropsis* can be achieved by homologous recombination [61•], thus allowing precise transgene targeting. It is expected that the genetic toolbox for microalgae will expand even further in coming years. In higher plants, a number of advanced genetic tools have been developed. These include synthetic promoters, tuneable transcription factors, genome-editing tools and site-specific recombinases [62]. One such method, using zinc-finger nucleases for the specific modification of endogenous nuclear genes has already been optimised for *C. reinhardtii* [63•]. With such microalgal genetic techniques continuing to progress, and the understanding of microalgal lipid metabolism developing rapidly, metabolic engineering could prove invaluable in the development of economically viable algal biofuels.

One enzyme family that has been identified as potentially the most promising target for over-expression are the DGATs [37, 64, 65]. DGATs have been studied considerably in higher plants and can be categorised into three subgroups: Type-1, Type-2 and Type-3, and they catalyse the final committed step in TAG biosynthesis [40]. The *C. reinhardtii* Type-2 DGAT genes have been shown in several transcriptomic studies to be up-regulated when TAG accumulation is induced under stress conditions [44, 47•, 66]. Furthermore, DGATs have been suggested to be the primary enzyme for de novo TAG biosynthesis in all organisms studied so far [67]. However, despite the evidence suggesting that Type-2 DGATs are key players in TAG accumulation, over-expression of these genes in *C. reinhardtii* had no effect on the levels of TAG or total lipids [68•]. The Type-2 DGAT may therefore be a downstream response to another enzyme, and there may be a bottleneck in carbon flux further back in the TAG biosynthesis pathway. This highlights the potential pitfalls

Table 1 Summary of selected genetic approaches for the manipulation of microalgae for improved biofuel characteristics

Trait	Process modified	Target	Method	Outcome	Species	Reference
Lipid yield	TAG catabolism	Lipase (Thaps3_264297)	RNA interference and antisense	2.4–3.3 fold increase (exponential growth); 3.2–4.1 fold increase (after Si starvation)	*Thalassiosira pseudonana*	[72••]
Lipid yield	TAG synthesis	Type-2 DGAT	Over-expression	35 % increase	*Phaeodactylum tricornutum*	[70]
Lipid yield and productivity	TAG synthesis	Unknown	Heavy-ion irradiation mutagenesis	14 % increase (TAG content); 28 % increase (lipid productivity)	*Nannochloropsis oceanica*	[89]
Lipid yield and productivity	Unknown, maybe TAG catabolism	Unknown but including long-chain fatty acid ligase	Ultraviolet mutagenesis	80 % increase (TAG productivity)	*Tisochrysis lutea*	[91, 92•]
Hydrogen production	Hydrogenase O_2 sensitivity	PsbO (PSII)	RNA interference	10 fold increase	*Chlorella* sp. DT	[74]
Hydrogen production	Thylakoid proton gradient	PGRL1	DNA insertional mutagenesis (random screen)	3 fold increase	*Chlamydomonas reinhardtii*	[75]
Hydrogen production	State transitions; increased PSII stability	STM6	DNA insertional mutagenesis (random screen)	5–13 fold increase	*C. reinhardtii*	[76•]
Hydrogen production	Photosynthetic efficiency; reduced antenna size	LHCBM1, 2, 3 light harvesting complex proteins	RNA interference	2 fold increase	*C. reinhardtii*	[77]
Growth rate (under high light)	Reduced antenna size; reduced photo-inhibition	All light-harvesting complex proteins	RNA interference	45 % increase after ~28 h	*C. reinhardtii*	[78]

DGAT diacylglycerol acyltransferase, *PSII* photosystem II, *TAG* triacylglycerol

of the metabolic engineering of intricate metabolic pathways in complex organisms. In contrast, one of the four Type-2 DGAT isoforms and a Type-3 DGAT from the diatom *Phaeodactylum tricornutum* were able to restore TAG biosynthesis when expressed in a TAG-deficient yeast strain [69, 70]. More recently, over-expression of a Type-2 DGAT in *P. tricornutum* led to a 35 % increase in neutral lipid accumulation [71]. However, it should be noted that only one over-expression line was presented in this study.

An alternative approach has been taken with another diatom species to reduce TAG catabolism. A TAG lipase was knocked down in *Thalassiosira pseudonana*, which led to a considerable increase in TAG content without any adverse effects on growth [72••] (Table 1). This result is extremely promising as it confirms that it is possible to increase TAG yields without negative effects on growth, and the findings also provide hope that further improvements in TAG yields can be made through manipulating other target genes. It is also possible that TAG yields may be increased further by using a combined approach of reducing TAG catabolism and increasing TAG anabolism simultaneously.

In addition to manipulating lipid metabolism, various transgenic approaches have been used to manipulate hydrogen production, mainly through RNA interference- or DNA insertional mutagenesis-based gene silencing [73] (Table 1). Some of these approaches include reducing the oxygen sensitivity of hydrogenases, such as through the mutation of photosystem II (PSII) components [74], manipulating the thylakoid proton gradient by mutation of cyclic electron flow components [75], manipulating the photosynthetic state transition by improved PSII stability by mutation of a state transition regulator [76•] or increasing the photosynthetic efficiency for increased hydrogen production by decreasing the chlorophyll antenna size of the photosystems by mutation of light-harvesting complex proteins to increase the light use efficiency [77]. Such manipulation of antenna size is not just of benefit for hydrogen production and has been shown to reduce photo-inhibition and increase the growth rate under high light conditions [78].

Natural Algal Strain Diversity and Non-genetic Manipulation Methods

Whilst a considerable focus has been placed upon GM approaches to increase oil yields and improve biomass productivity, it is important not to forget the substantial possibilities that non-GM approaches provide, in particular because of the regulatory, commercial and ethical constraints of GM organisms, plus the potential environmental safety implications. A challenge with any GM strain is containment, both to prevent

loss into the environment and mitigation of unknown consequences including the risk of transgene flow, and the loss of the GM strain in the mass culture through contamination with non-GM strains or predation. A mechanistic model was used to simulate the consequences of GM strains with reduced photosystem antenna size and modified photosynthetic efficiency, which would be expected to perform better in mass cultivation conditions. It was predicted that such strains would have reduced evolutionary fitness and be disadvantaged in competition with non-GM strains [79]. However, modelling of a GM strain with various biofuel optimisation traits, including growth rate, respiration, and nutrient use, suggests that such a strain will less likely be grazed upon by a predator than a non-GM strain and therefore would have increased risk of generating harmful algal blooms in natural environments [80•].

Screening natural strains for suitable characteristics including high biomass productivity or oil yield either from culture collections or from natural environments (bioprospecting) is another approach. Only a handful of microalgal species are being characterised in depth for biofuel development or used as 'model' species, including *Nannochloropsis* sp., *Dunaliella* sp., *Chlorella* sp., *C. reinhardtii*, *B. braunii*, *T. pseudonana*, *P. tricornutum* and *Tisochrysis lutea*. In contrast, many thousands of microalgal species and strains are likely to exist with desired characteristics. Even through screening of small numbers of strains (<100), it is apparent that there are marked variations in total lipid yield, fatty acid characteristics and growth rate [49, 81]. Bioprospecting studies are continuing to identify useful natural strains [82–84]. Ideally, such studies depend on accurate but high-throughput screening techniques such as flow cytometry or vibrational spectroscopy that can rapidly screen metabolite characteristics [84–87], but also genomics techniques for strain identification and cryopreservation methods [42]. Characteristics from natural strains could be used to improve and combine particular characteristics in commercially useful biofuel strains, such as through marker-assisted breeding. Quantitative trait loci (QTLs) and rare alleles associated with a desirable trait and could be bred into commercial strains of microalgae. Although this approach has not been studied extensively in algae, a method such as EcoTilling [88] could be used to identify the naturally occurring alleles of, for example, lipid metabolism genes. Such preferred alleles could be subsequently bred into commercial strains of microalgae to obtain high levels of TAG accumulation. In addition, genetic information from natural strains could be an important resource for GM of commercial strains.

Another approach of strain modification that is often not regarded as GM, because transgenic material is not involved, is mutagenesis screening (forward genetics) performed using an exogenous mutagen such as ultraviolet light. Mutagenesis screens could generate commercial algal strains without the need for a targeted approach, and could also identify novel genes previously unknown to be involved in lipid metabolism. Novel methods of mutagenesis [89] and screening [87, 90, 91] have been recently developed that could be used across a large number of oleaginous microalgal species, including less widely studied species, to improve characteristics for biofuel production. One study, using heavy-ion irradiation mutagenesis identified a *Nannochloropsis oceanica* mutant with a 28 % increase in lipid productivity and improved growth characteristics [89]. In another study, a strain of *T. lutea* was mutated by two rounds of ultraviolet treatment and flow cytometry selection to yield an 80 % increase in neutral lipid productivity [91]. Subsequent analysis of this strain by transcriptome sequencing found that 291 transcripts were differentially expressed, 165 of which contained a single nucleotide polymorphism. Although the majority of these transcripts were not known lipid metabolism genes, one encoded a putative long-chain fatty acid ligase required for the esterification of free fatty acids, which could potentially cause a defect in lipid catabolism [92•].

Conclusions

It is clear that there is a long way to go before the commercial-scale production of biofuels from algal feedstock will be a reality. Although production of algal biodiesel via heterotrophic fermentation can be performed on a commercial basis [18], there are doubts regarding the economic sustainability and the scalability of this method of production. There are currently a large number of factors that have to be overcome both to improve the techno-economic feasibility of the process and to alleviate some of the sustainability concerns, particularly with regard to the excessively high amounts of fertilisers that are estimated to be required. However, progress in the identification and development of new strains, particularly of microalgae, that are better tailored for the mass cultivation of biofuel products is promising. In addition, some of the significant improvements in our understanding of the fundamental biological processes of algae and the proof-of-concept developments through genetic manipulation are providing positive outcomes. In the meantime, commercial use of algae will likely increase for the development of high-value products and applications, and knowledge gained from this experience may be beneficial to future cultivation of algae for fuel products.

Although basic research should continue to be directed towards strain development, not just for improved biomass production and oil yield, but for more efficient input (e.g. fertiliser) use, the use of such strains, particularly if they are generated by GM or synthetic biology technologies, must controlled by appropriate regulation. There is a strong potential that useful genetic mutation identified in the laboratory can be transferred into commercial strains using non-GM approaches such as ultraviolet mutagenesis and tilling, and therefore such non-GM strains would be more attractive to industry. However, potential environmental impacts of all novel strains must be rigorously assessed. Research is also needed to assess the potential

applications of by-products from algal biomass following extraction of biofuel products, to further exploit the economic value of the biomass (biorefining of algae biomass). For example, following extraction of algal oil, some of the residues may be used for the isolation of useful chemicals or used as a biofertiliser or animal feed, if the residue biomass is nutrient rich. Alternatively, the remaining biomass may be further converted into bioenergy products such as via thermochemical conversion or anaerobic digestion. The choice of biomass use and the development of particular products will be largely determined by market price. However, robust LCA and techno-economic analysis must also be further developed and used to make sure that the future algal biofuel and biotechnology industries are both economically and environmentally sustainable. All of these developments must be evaluated under scaled-up conditions to demonstrate commercial viability, and there are many engineering challenges to be overcome to allow efficient, low-cost and low-energy algal cultivation on a sufficiently large scale. Finally, there needs to be both industry and governmental policies in place to support the use of algae as a feedstock for bioenergy and transportation fuels, not just through public funding to support the various research and development needs, but potential legislative or tax incentives for encouraging the commercial development and use of algal-derived fuels. The development and implementation of international biofuel standards will also be important in the adoption of biofuels in general, and the acceptance of algal-derived biofuels in particular. Currently, crop-derived bioethanol from Brazil and the USA accounts for the majority of the global biofuel sector. Ultimately, the diversification of biofuel sources is important to improve energy security and environmental sustainability, and algae biofuel could be a major component in a diverse bioenergy sector.

Compliance with Ethics Guidelines

Conflict of Interest Thomas Driver, Amit Bajhaiya and Jon K. Pittman declare no conflict of interest.

Human and Animal Rights and Informed Consent The manuscript does not contain clinical studies or patient data.

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- •• Of major importance

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