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Second Generation Biofuels: High-Efficiency Microalgae for Biodiesel Production

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Abstract The use of fossil fuels is now widely accepted as unsustainable due to depleting resources and the accumulation of greenhouse gases in the environment that have already exceeded the "dangerously high" threshold of 450 ppm CO_2-e. To achieve environmental and economic sustainability, fuel production processes are required that are not only renewable, but also capable of sequestering atmospheric CO_2. Currently, nearly all renewable energy sources (e.g. hydroelectric, solar, wind, tidal, geothermal) target the electricity market, while fuels make up a much larger share of the global energy demand (~66%). Biofuels are therefore rapidly being developed. Second generation microalgal systems have the advantage that they can produce a wide range of feedstocks for the production of biodiesel, bioethanol, biomethane and biohydrogen. Biodiesel is currently produced from oil synthesized by conventional fuel crops that harvest the sun's energy and store it as chemical energy. This presents a route for renewable and carbon-neutral fuel production. However, current supplies from oil crops and animal fats account for only approximately 0.3% of the current demand for transport fuels. Increasing biofuel production on arable land could have severe consequences for global food supply. In contrast, producing biodiesel from algae is widely regarded as one of the most efficient ways of generating biofuels and also appears to represent the only current renewable source of oil that could meet the global demand for transport fuels. The main advantages of second generation microalgal systems are that they: (1) Have a higher photon conversion efficiency (as evidenced by increased biomass yields per hectare): (2) Can be harvested batch-wise nearly all-year-round, providing a reliable and continuous supply of oil: (3) Can utilize salt and waste water streams, thereby greatly reducing freshwater use: (4) Can couple CO_2-neutral fuel production with CO_2 sequestration: (5) Produce non-toxic and highly biodegradable biofuels. Current limitations exist mainly in the harvesting process and in the supply of CO_2 for high efficiency production. This review provides a brief overview of second generation biodiesel production systems using microalgae.

Keywords Algae · Carbon sequestration · Biofuel · Biogas · Biohydrogen · Biomethane · Bioreactor · Lipid · Oil · Raceway pond · Triacylglycerides · Review

Abbreviations

BTL	biomass to liquid
CFPP	cold filter plugging point
CO_2-e-CO_2	equivalents of greenhouse gases
NEB	net energy balance
LHC	light harvesting complex
OAE	oceanic anoxic event
PS	photosystem

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RuBP	ribulose-1,5-bisphosphate
Rubisco	ribulose 1,5 bisphosphate carboxylase/ oxygenase
TAG	triacylglycerides

Significance of Biodiesel Production from Algae

Introduction

The development of CO_2-neutral fuels is one of the most urgent challenges facing our society. In the last 18 months, this fact has been brought into sharp focus by the Stern Report 'The Economics of Climate Change' [170] and the Intergovernmental Panel on Climate Change 'AR4 Synthesis report' [84]. Together these reports provide the most comprehensive evaluation of the causes and effects of climate change currently available. Importantly they define atmospheric CO_2 levels above 450 ppm CO_2-e (i.e. e = equivalent contribution of all greenhouse gases) as already being in the dangerously high range and conclude that we exceeded this threshold (currently 455 ppm CO_2-e) 10 years earlier than had previously been predicted.

These new and startling conclusions are leading governments to establish CO_2 emission reduction targets which typically lie in the range of 10–20% by 2020 (e.g. European Union). Germany recently suggested a 30% cut by 2020 if matched by other nations. Yet these proposed emission reductions are not sufficient to stabilize CO_2 levels in an accepted 'safe zone' (i.e. below 450 ppm CO_2-e; IPCC [84]). Indeed they are more likely to lead to stabilization levels above 550 ppm. At these levels much more destructive effects are predicted [84]. So, although a range of CO_2 reduction scenarios to stabilize emissions have been developed, the IPCC concluded that to stabilize in a range of 445–490 ppm of atmospheric CO_2-e, emissions must peak before 2015 and a total CO_2 reduction of 50–85% will be required by 2050. Importantly, the IPCC indicates that this might be an underestimate due to missing carbon cycle feedback effects in the current climate change models. Consequently more stringent emission reductions (e.g. 60% by 2020) are increasingly thought to be more appropriate and such concern has led Rajendra Pachauri, the chairman of the IPCC to state, "What we do in the next 2 or 3 years will define our future" [126].

Achieving a 60% CO_2 emission reduction by 2020 or even a 50–85% cut by 2050 is, however, an enormous global challenge which will require the development of a suite of renewable energies. There is now a concerted effort to develop new biofuels, of which biodiesel and bioethanol are seen as being close to market options. The areas of biomethane, biomass-to-liquid (BTL)-diesel and biohydrogen are also developing rapidly.

This review provides a brief overview of the background and recent developments in second generation microalgal systems with a specific focus on biodiesel, which have the potential to couple renewable, CO_2-neutral fuel production with power plant flue and atmospheric CO_2 sequestration. Specifically, it aims to define the current state of the art, and to identify major opportunities for future innovation in the biodiesel field.

Biofuels and Biodiesel in the Global Context

The global energy market can essentially be divided into the electricity and fuel sectors. Both sectors will have to achieve significant emission reductions to meet planned international legislated targets. Currently, the electricity sector accounts for approximately 33% of global energy and is developing a range of low CO_2-emission approaches for electricity production (i.e. nuclear, solar, wind, geothermal, hydroelectric, clean-coal technology). In contrast, fuels accounted for a much larger (~67%) market share of the global energy consumption ~15.5 TW (489 EJ/year) in 2005 according to the Energy Information Administration, USA. Yet, despite the obvious importance of fuels, CO_2-neutral (e.g. biodiesel, bioethanol, biomethane, BTL-diesel) and CO_2-free (e.g. biohydrogen) fuel production systems are far less developed. The range of biofuels available demonstrates the flexibility and potential of the biofuel industry. However, this potential was not fully realized by the first generation biofuel systems due to serious economic and environmental limitations (see "Addressing the Concerns of Biofuel Production" and "Economic Feasibility of Microalgal Biodiesel" sections). In contrast, second generation biofuel systems (such as lignocellulosic and microalgal biofuel systems) have the potential to overcome many of these limitations and target a newly emerging clean energy market which is predicted to expand rapidly to a value of US $500bn by 2050 [170], or more (current oil market value is US $2.65 Trillion).

By far the largest proportion of biofuels is produced from higher plants which use photosynthesis to convert solar energy into chemical energy (Fig. 1). In nature, this chemical energy is stored in a diverse range of molecules (e.g. lignin, cellulose, starch, oils). Lignocellulose, the principle component of plant biomatter, can be processed into feedstock for ethanol production. This can be achieved by either gasification or by cellulolysis (chemical or biological enzymatic hydrolysis). These processes are currently being developed for second generation biofuel systems [41, 155] and are often referred to as 'lignocellulosic processes'. Similarly, starch (e.g. from corn) and sugar

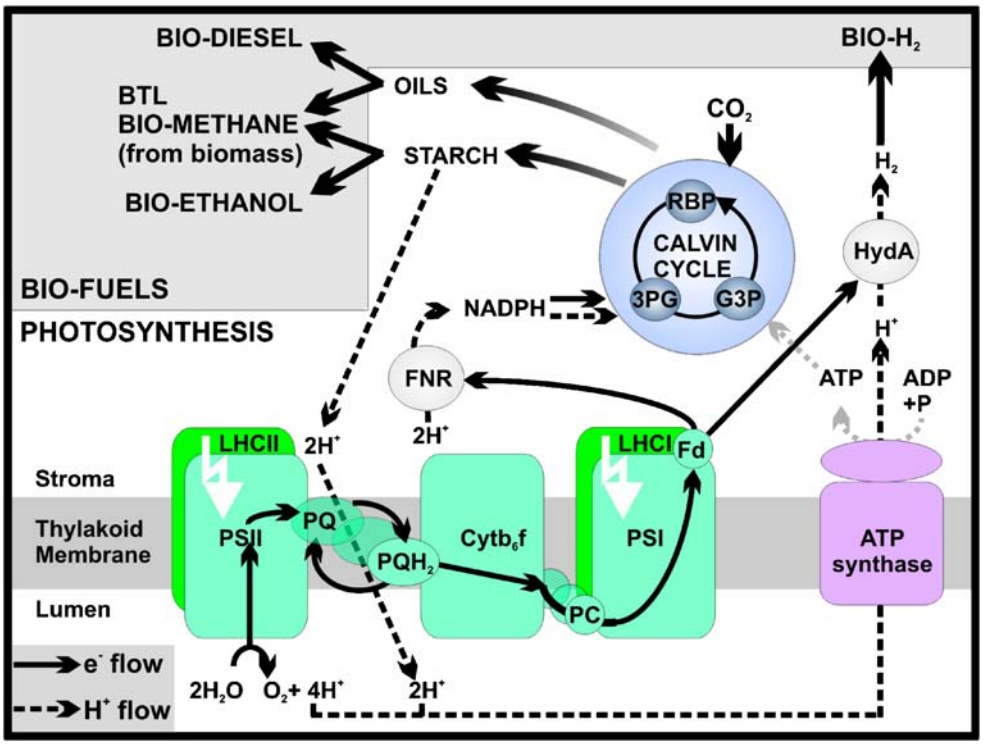

Fig. 1 The process of photosynthesis coverts solar energy into chemical energy and is key to all biofuel production systems in plants

(e.g. from sugarcane) are already being converted into bioethanol by fermentation [23, 74], while oils (e.g. from canola, soy and oil palm) are being used as a feedstock for the production of biodiesel [80, 156]. Microalgae are able to efficiently produce cellulose, starch and oils in large amounts [16, 162]. In addition, some microalgae and cyanobacteria (which produce glycogen instead of starch) can also produce biohydrogen under anaerobic conditions [20, 35, 59, 76, 111] and their fermentation can also be used to produce methane (Fig. 1; "Other Biofuels from Microalgae" section).

The Flexibility of Biofuel Production Systems

Central to all light-driven biofuel production is the process of photosynthesis. It is the first step in the conversion of light to chemical energy and ultimately responsible for driving the production of the feedstocks required for a wide range of fuel synthesis (Fig. 1): protons and electrons (for biohydrogen), sugars and starch (for bioethanol), oils (for biodiesel) and biomass (for BTL, biomethane).

In higher plants and green algae, light is captured by specialized light harvesting complex proteins, referred to as LHCI and LHCII (Fig. 1). These are encoded by a large gene family that exhibit a high degree of homology [51] and their expression is dependent on the prevailing environmental condition (e.g. light intensity). These proteins bind the bulk of the chlorophyll and carotenoids in the plant and play a role both in light capture and in the dissipation of excess energy which would otherwise inhibit the photosynthetic reaction centres, in particular photosystem II (PSII; [81]). Excitation energy used to drive the photosynthetic reactions is funnelled to the photosynthetic reaction centres of photosystem I (PSI) and PSII via the highly coordinated network of pigments bound by the LHC, PSII and PSI subunits. In the first step PSII uses this energy to drive the photosynthetic water splitting reaction, which converts water into protons, electrons and oxygen. The electrons are passed along the photosynthetic electron transport chain via plastoquinone (PQ), cytochrome b_6f (Cyt b_6f), photosystem I (PSI), and ferredoxin (Fd) and on to NADPH (Fig. 1). Simultaneously, protons are released into the thylakoid lumen by PSII and the PQ/PQH_2 cycle. This generates a proton gradient, which drives ATP production via ATP synthase. The protons and electrons are recombined by ferredoxin-$NADP^+$ oxidoreductase (FNR) to produce NADPH. NADPH and ATP are used in the Calvin cycle and other biochemical pathways to produce the sugars, starch, oils and other bio-molecules (which collectively form biomass) that are required to produce bioethanol, biodiesel, biomethane and BTL-based biofuels. Alternatively in some photosynthetic microorganisms like the green alga *Chlamydomonas reinhardtii*, the protons and electrons extracted from water (or starch) can be fed to the hydrogenase enzyme (HydA) via the electron transport chain to drive the direct photo-production of biohydrogen (Fig. 1). The production of

biohydrogen by the algae and biomethane from biomass as a downstream product are briefly discussed in the section on "Other Biofuels from Microalgae".

The Calvin cycle is an integral part of the photosynthetic process and responsible for fixing CO_2, in a diverse range of organisms including primitive algae through to higher plants. The process uses ATP and NAD(P)H generated by the light reactions. In C4 and CAM plants it is coupled to ancillary processes that aid CO_2 fixation, but the fundamental photosynthetic reduction cycle reactions remain the same [175]. The Calvin cycle can be divided into three main steps that involve carboxylation, reduction and substrate (ribulose-1,5-bisphosphate (RuBP)) regeneration. The first step at which CO_2 enters the cycle to react with RuBP is catalyzed by ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco). The importance of rubisco is hard to overstate as essentially all carbon found in living organisms on Earth was once fixed by this enzyme from atmospheric CO_2. Furthermore it is the most abundant protein on Earth, constituting some 30% of total proteins in most leaves [129]. This is partly because of its central role for photosynthesis, but also because it has a very low catalytic carboxylase performance, using as little as 2–3 RuBP per second [104]. As its name suggests rubisco has two catalytic functions; it functions as a carboxylase as part of the photosynthetic reduction cycle, and under aerobic conditions as an oxygenase as part of photorespiration. O_2 and CO_2 compete for the same catalytic site, so that the efficiency of CO_2 fixation can be impaired in certain aerobic environments. For example, although the specificity of the enzyme is higher for CO_2 (e.g. tobacco (higher plant) 82 times, *Griffithsia monilis* (red alga) 167 times, *Rhodospirillum rubrum* (purple non-sulfur bacteria) 12 times [7]), the molecular ratio of O_2/CO_2 is about 540:1 in air and 24:1 in air-saturated water at 25°C. In the first step of the Calvin cycle, rubisco catalyzes the formation of two 3-phosphoglycerate molecules from RuBP, CO_2 and H_2O. The forward reaction is strongly favoured by the negative change in free energy of the process. In the second step, an ATP/NADPH-dependent reduction phase, these carboxylic acids are reduced to form two molecules of glyceraldehyde-3-phosphate, by the action of phosphoglycerate kinase and glyceraldehyde-3-phosphate dehydrogenase. In a third step, consisting of a series of reactions a proportion of glyceraldehyde-3-phosphate is converted back to RuBP required to allow the photosynthetic reduction cycle to continue [175].

Addressing the Concerns of Biofuel Production

Although biofuel processes have a great potential to provide a carbon-neutral route to fuel production, first generation production systems have considerable economic and environmental limitations and recently, the issue of biofuels has become a hot debate. While benefits of biofuels are emphasized by some [157, 167], others have criticized the economics and carbon mitigation potential of biofuel production ([19, 128, 131, 147, 190]).

The most common concern related to the current first generation of biofuel systems is that as production capacities increase, so does their competition with agriculture for arable land used for food production. For example, current biodiesel supplies from oil-producing crops, supplemented with small amounts of animal fat and waste cooking oil, only account for an estimated 0.3% (approx. 12 million tons in 2007) of the current global oil consumption [24, 125] and can not even come close to satisfying the existing and future demand for transport fuels. Currently approximately 8% of plant-based oil production is used as biodiesel [125] and this has already contributed to an increase of the price of oil crops over the last few years.

Area Requirements for Biofuel Production It is estimated that the surface of the Earth (510,072,000 km^2) receives ~170 W m^{-2} of solar power on average [31, 196]. This equates to 2.735 YJ of energy per year and corresponds to ~5,600 times the global consumption of primary energy in 2005 (488 EJ; [196]). Consequently the solar energy required to produce biofuels is available in abundance. However, even if current oil-producing crops would be grown on all arable land (assuming 29.2% of the Earth is land of which 13% are arable, energy conversion efficiencies of 1% from sunlight to biomass, and 20% yield as oil), these would be able to cover less than half of our energy demand today. Using such conservative figures, biofuel critics have often concluded that biofuel production can not contribute in any major way to global fuel requirements. However as will be highlighted here, much higher photosynthetic efficiencies and oil yields are already achievable and so open up second generation biofuel technologies with enormous potential.

The increased pressure on arable land has already resulted in considerable problems and unsustainable practises worldwide that have led to the coining of the phrase "peak soil". For example, rainforest regions in Brazil and South East Asia are currently being cleared at an unprecedented rate to make room for soybean and oil palm plantations for the production of biodiesel. Annual biodiesel production growth rates in Indonesia, Malaysia and Thailand are currently between 70% and 250% [181]. The increased pressure on arable land currently used for food production could lead to severe food shortages, in particular for the developing world where already more than 800 million people suffer from hunger and malnutrition (figure without China; [53]). In addition, the intensive use of land with high fertilizer and pesticide

applications and water use can cause significant environmental problems.

Net Energy Balance When evaluating the value and sustainability of a biofuel production process it is necessary to establish its Net Energy Balance (NEB). The NEB of first generation biofuels taking into consideration the energy required for farming, harvesting, processing, transport, etc. has recently been estimated to be ~25% for corn ethanol and + ~93% for soybean biodiesel [80], though precise values are dependent on detailed case by case life cycle analyses. While this report counters claims that energetic costs of fertilizer applications, farming machinery and processing facilities cause negative NEB values for both biofuels, it does not as yet factor in projections by the Intergovernmental Panel on Climate Change that traditional crop yields could fall up to 50% by 2020 [84].

Carbon Balance Calculating the carbon balance of a process is equally important. Biofuel production from crops is often estimated to be a near carbon-neutral process as nearly all carbon in conventional biofuel crops is derived from atmospheric CO_2, which is released on combustion. However to be precise the overall CO_2 emissions balance for biofuel production, must also be evaluated on an individual production case basis and include energy-intensive fertilizer production, the use of machinery for cultivation and refinery, and transport which are processes that currently emit fossil fuel-derived CO_2. Furthermore, the production of oil from oil palm plantations established prior to the legislation of Kyoto emissions targets, is considered to lead to improved reductions of emissions compared to conventional diesel. In contrast however, if rainforest regions must first be cleared to make room for the plantation, the CO_2 emissions balance is exceedingly poor [14].

CO_2 Sequestration One important development which is predicted not only to greatly improve the net CO_2 balance of biofuel processes, but actually contribute to atmospheric CO_2 reductions, is the coupling of biofuel production to CO_2 sequestration systems. These typically involve the production of Agri-char by a process of pyrolysis (see section on "CO_2-Sequestration").

Second Generation Biofuel Systems A range of second generation of biofuel-production systems that will have much higher NEBs, are more water-efficient and require much less arable land are now under development. Of particular interest are lignocellulosic technologies and microalgae ([29, 76, 96, 155]; Table 1). Microalgae are already reported to produce 15–300 times more oil for biodiesel production than traditional crops on an area basis (Table 1; [29]). Furthermore compared with conventional crop plants which are usually harvested once or twice a year, microalgae have a very short harvesting cycle (~1–10 days depending on the process), allowing multiple or continuous harvests with significantly increased yields (Table 1). Greater light capture and conversion efficiencies ultimately lead to reduced fertilizer and nutrient inputs and so result in less waste and pollution. The use of waste water for algal cultivation is also a viable option ([75, 103, 120]; see "Using Wastewater and Seawater Resources" section). Furthermore as the cultivation of microalgae for biofuel production can potentially be carried out on marginal or non-arable land, this could further reduce the competition for land and open up new economic opportunities for arid,

Table 1 Comparison of crop-dependent biodiesel production efficiencies from plant oils

Plant source	Biodiesel (L/ha/year)	Area to produce global oil demand (hectares × 10^6)	Area required as percent global land mass	Area as percent global arable land
Cotton	325	15,002	100.7	756.9
Soybean	446	10,932	73.4	551.6
Mustard seed	572	8,524	57.2	430.1
Sunflower	952	5,121	34.4	258.4
Rapeseed/canola	1,190	4,097	27.5	206.7
Jatropha	1,892	2,577	17.3	130 (0[a])
Oil palm	5,950	819	5.5	41.3
Algae (10 g m^{-2} day^{-1} at 30% TAG)	12,000	406	2.7	20.5 (0[a])
Algae (50 g m^{-2} day^{-1} at 50% TAG)	98,500	49	0.3	2.5 (0*)

Presented yields are for peak performing crops [16, 29, 162], although for example, Malaysia's average oil palm yield is actually about 4 tons/ha [119]. Algae yield scenarios are based on existing production systems and their potential [16, 162]. Current algal production systems fall between these ranges: Seambiotic Israel (currently at 20 g m^{-2} day^{-1} at 8–40% Triacylglycerides (TAG), HR BioPetroleum Inc Hawaii (aims to achieve 50 g m^{-2} day^{-1} at 30% TAG)

[a] If algal ponds and bioreactors are situated on non-arable land; jatropha is mainly grown on marginal land.

drought or salinity-affected regions. In addition, while traditional biofuel crops require substantial amounts of fresh water, considerable savings in net water use can be achieved if closed bioreactor systems with minimal evaporation are used for algal cultivation, especially using marine and halophilic strains of microalgae. The microalgal biomass generated in bioreactors can also be gasified or pyrolysed to produce a range of biofuels and Agri-char as part of a CO_2 sequestration strategy. In this way biological GMO waste can also be effectively disposed of in an environmentally sensitive way. The carbon-rich biomass pellets can also be stored as part of a carbon sequestration strategy which could utilize CO_2 from power plants as an input for biomass production (see "CO_2-Sequestration" section).

Other significant aspects of second generation microalgal systems are that they are amenable to highly innovative biotechnology approaches that have the potential for rapid improvements of algal strains. These aspects promise increases in yield by metabolic engineering (see "Photosynthetic Efficiency" section). Recent advances have been made in many areas (see "Recent Advances in Microalgal Production" section), and examples of increased photosynthetic efficiency and tolerance to grow in saline or waste water streams are described in the "Using Wastewater and Seawater Resources" section. Some limitations of second generation microalgal systems have been identified in the harvesting process and the supply of CO_2 for high efficiency production (see "Cultivation of Microalgae" and "Recent Advances in Microalgal Production" sections).

Economic Feasibility of Microalgal Biodiesel

The potential of biofuel production is highlighted by the fact that even using current technology, Brazilian sugarcane-based and US corn-based bioethanol production are reported to be cost competitive at oil prices of US $40 and US $60 per barrel, while the equivalent for biodiesel is US $80 [178]. Thus, since the oil price has recently reached US $100 per barrel, these fuels can in theory already be produced economically Therefore, even modest increases in photosynthetic and process efficiency are expected to yield significant increases in economic competitiveness. Furthermore oil production (which has driven up the oil price) is increasingly thought to be close to its maximal capacity, indicating that additional bio-fuel production streams will increasingly find a market.

Biodiesel Biodiesel is developing into one of the most important near-market biofuels as virtually all industrial vehicles used for farming, transport and trade are diesel-based. In the past decade, the biodiesel industry has seen massive growth globally, more than doubling in production every 2 years [125]. The increased demand for vegetable oils for the production of biodiesel has led to significant pressure on the vegetable oil market. Indeed the world's biodiesel industry is currently operating far below capacity due to a lack of feedstock.

Second generation microalgal systems are increasingly predicted by international experts and policy makers to play a crucial role in a clean environmentally sustainable future as they have important advantages. Most significantly, these achieve a higher yield per hectare (potentially over 15-fold higher than oil palm, the biggest current oil producer; Table 1) and their ability to be cultivated on non-arable land, thereby reducing the competition with food crops for land (see "Addressing the Concerns of Biofuel Production" section). Algal production systems are recognized as among the most efficient means of producing biomass for fuel (Table 1; [16, 29, 162]) and further improvements are likely to occur in the near future. For example, algae are already being engineered for increased photosynthetic efficiency of the overall culture due to improved light penetration and reduced fluorescence and heat losses (see "Photosynthetic Efficiency" section).

A number of studies have attempted to calculate the cost of algal oil production from large scale farms. A comprehensive analysis and a good road map for building such systems [16] estimated the cost of algal oil to be in the range of $39–$69 US/barrel (bbl), accounting for inflation to 2008 this would equate to $52–$91 US/bbl. This estimate was based on 400 hectares of open ponds, using either pure CO_2 or flue gas from a coal-fired power station and productivity assumptions of 30–60 g m^{-2} day^{-1} with 50% algal lipid yield. Such high yields are theoretically possible but to date have not been demonstrated. A more recent analysis [83] estimated algae oil production costs to be $84 US/bbl. This scenario was based on the infrastructure cost assumptions by Benemann and Oswald [16] but utilized a hybrid system ("Hybrid Systems" section) with an aerial productivity of 70.4 g m^{-2} day^{-1} and 35% algal lipid yield. Companies commercially producing algae have not been as optimistic in the production costs. One such company that is close to achieving these values is Seambiotic Ltd. (Israel). It estimates production costs of its dried algae to be at $0.34 US/kg [3]. The productivity was 20 g m^{-2} day^{-1} and the total lipid content ranged from 8–40%. Assuming the average yield of 24% lipid, extracted at no further cost, then this equates to $1.42 US/kg for lipids which equates to $209 US/bbl. At the more optimistic 40% yield, lipids cost $0.85 US/kg which equates to $126 US/bbl. Calculations were based on the specific gravity of soybean oil (0.925 kg/L; 1 kg=1.08 L; 147.2 kg=1 bbl; [92]). Given that oil prices seem to be stabilizing around the $90 per barrel mark, some of these estimates suggest that microalgal biodiesel produc-

tion could already be economically viable. Clearly, the field is in its infancy and there is much potential to reduce costs and improve efficiency ("Cultivation of Microalgae" and "Molecular Improvements of Microalgae for Increased Biodiesel Yields" sections). Furthermore, industrial feasibility studies vary enormously on the precise parameters used. For example, incorporating improved efficiencies based on optimal bioreactor depth, or incorporating the economies of scale of bulk nutrient costs, have major effects on the calculated profitability of the plant. Simply doubling the operation of a plant from 5 to 10 years theoretically doubles the output at minimal additional capital cost. The conservative conclusion therefore is that using existing low-tech approaches, microalgal biodiesel production systems may already be economically viable.

Lipid Production by Microalgae in Nature

In nature, microalgal accumulation of lipids increases under certain conditions, thus when selecting and improving algae for high biodiesel production it is important to keep the factors in mind that lead to a natural accumulation of lipids in algae. Microalgae are known to grow more abundantly in nutrient-rich (eutrophic) waters leading frequently to algal blooms [127, 144, 160]. Once the algal population reaches its limits (either due to nutrient depletion or high cell densities that limit light penetration) a large number of the algal cells die. These are then heterotrophically degraded by microorganisms that require a large amount of oxygen. This results in an upwards shift of the anaerobic zone in the water (Fig. 2) and can in extreme cases lead to complete anaerobiosis with devastating consequences [127, 144, 160]. These consequences include the death of all higher organisms (plants and animals) that require oxygen for survival and the emission of potent greenhouse gases such as CH_4 and N_2O. Repeatedly throughout history these phenomena have also been paralleled on a greater scale by oceanic anoxic events (OAEs), where below surface levels, the oceans became completely depleted of oxygen on a global scale. OAEs [9, 48, 106] are reported to result from previous global warming cycles where increased temperatures and CO_2 levels both contribute to their manifestation. It has been proposed that some of the world's largest oil deposits were actually formed as a result of algal carbon deposition during OAEs.

The trophic status of lakes has been defined based on primary production and can be measured by the amount of organic carbon assimilated by photosynthesis. Rodhe [149] defined the following scale: oligotrophic 7–25 g C m^{-2} $year^{-1}$; eutrophic (natural or mesotrophic) 75–250 g C m^{-2} $year^{-1}$; eutrophic (polluted) 350–700 g C m^{-2} $year^{-1}$. Oligotrophic or mesotrophic aquatic systems lead to initial algal growth until growth-limiting nutrients (typically N or P) become scarce. However if the cell density is not too high, photosynthesis is still possible. While actual growth is limited under these conditions, cells are still able to fix CO_2 and accumulate photo-assimilates in the form of starch or lipids that have important storage functions for survival under unfavorable conditions (e.g. low nutrients, micro-oxic conditions, anaerobiosis, low/high light or dry, hot or cold conditions). Algae that accumulate lipids in large amounts are therefore often found where environments and microclimates alter frequently between optimal growth conditions and survival under suboptimal conditions. Under normal growth conditions most algal species have a lipid content of ~10–30% dry weight. However, during nitrogen depletion the cells stop dividing and the storage products continue to accumulate at approximately the same rate as in non-nitrogen limited cells [162] with the result that dry weight lipid (hydrocarbon) contents can double or triple (e.g. *Botrytococcus braunii* 25–

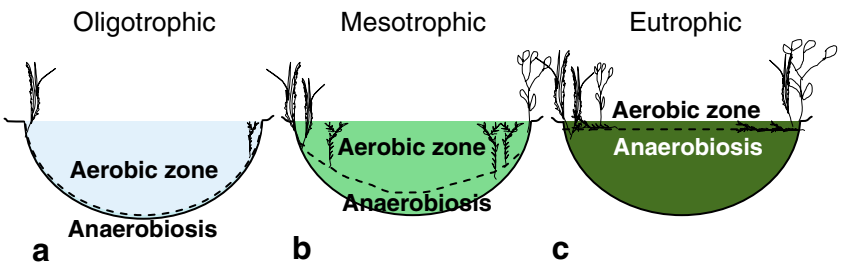

Fig. 2 Trophic states of aquatic systems and lipid producing algae. Depending on the level of available organic carbon, aquatic systems are classified as oligotrophic (**a**), mesotrophic (**b**) or eutrophic (**c**). Oligotrophic aquatic systems are usually deep, receive nutrient-poor water and little organic matter is produced that could sink and consume oxygen on decomposition during summer. In contrast, eutrophic aquatic systems are usually shallow, their drainage basin is richer, and rivers and ground-water discharges contain more nutrients. In addition primary production of plants, algae and bacteria is higher and as a result more organic material sinks and decomposes leading to depletion of oxygen. The consequence is that the deep water and sometimes the entire aquatic system can become anoxic during summer. For lipid-producing algae, oligotrophic or mesotrophic aquatic systems typically lead to initial algal growth followed by a lipid accumulation phase when nutrients are scarce but photosynthesis is still possible. Lipid-producing algae species can also be found in large numbers in meso- or eutrophic waters, but their lipid accumulation will not occur until nutrients become scarce, under adequately illuminated conditions that support photosynthesis and so maintain an aerobic state

75%, *Nannochloropsis* sp. 31–68%, *Schizochytrium* sp. 50–77%, *Neochloris oleaabundans* 35–54%, *Nitschia* sp. 45–47%; [26, 29, 114, 168]).

Other Biofuels from Microalgae

Apart from high efficiency production of oil for biodiesel, microalgae are also well suited for the production of feedstocks for other biofuels. The development of the technologies for high efficiency algal biodiesel production is also applicable to biohydrogen, biogas, bioethanol and biomass-to-liquid (BTL) approaches using fast growing algae. BTL, biohydrogen and biomethane processes are discussed below as they are especially pertinent to microalgal system. Bioethanol is not described here as the processes used for its production with microalgae as feedstocks are very similar to established 1st generation technologies that use corn- and sugarcane-derived feed stocks.

Algal Biohydrogen Production

Microalgae for photo-biological hydrogen production from water are being developed into a promising and a potentially emission-free fuel stream for the future, which could also be coupled to atmospheric CO_2-sequestration. Biohydrogen production from microalgae has been known for more than 65 years and was first observed in the green alga *Scenedesmus obliquus* [59] and later identified in many other photosynthetic species including cyanobacteria [20, 35]. Most studies on algal hydrogen production have been performed using the green alga *Chlamydomonas reinhardtii,* a model organism for photosynthesis research [78, 148], via an aerobic-anaerobic cycle developed by Melis and coworkers in 2000 [63, 111]. The bio-H_2 process is attractive in that it uses sunlight to convert water to hydrogen and oxygen, which are released in a two-phase process through the following reactions:

$$H_2O \rightarrow 2H^+ + 2_e^- + {}^1/_2O_2$$
$$2H^+ + 2_e^- \rightarrow H_2$$

The first reaction occurs in all oxygenic photosynthetic organisms while the second reaction is mediated by special iron containing chloroplast-hydrogenase enzymes and is restricted to a more select group of microalgae [57, 77]. Cyanobacteria also have the ability to produce H_2 from water but use an alternative biochemical process. Under normal light and aerobic conditions, H^+ and e^- from the water-splitting reaction of photosynthesis are used for synthesis of ATP and NADPH.

The second reaction occurs under anaerobic conditions. In the absence of O_2 both ATP production by oxidative phosphorylation, and the formation of NADH/NADPH are inhibited [67]. Under these conditions, certain microalgae, such as *C. reinhardtii* reroute the energy stored in carbohydrates such as starch to the chloroplast hydrogenase [63, 111] to facilitate ATP production via photophosphorylation and to keep the electron transport chain from over-reduction (see [96, 151] for a review; Fig. 1). Thus, hydrogenase together with different fermentative processes [93] essentially acts as a proton/electron release valve by recombining protons from the medium and e^- from reduced ferredoxin to produce hydrogen gas that is excreted from the cell [111]. *C. reinhardtii* therefore provides the basis for solar-driven bio-H_2 production from water and can also use other fermentative processes. A major advantage of hydrogen production is that hydrogen does not accumulate in the culture but is quickly released into the gas phase, unlike other fermentative products, which can build up to levels toxic to the cells.

Recently progress has been made towards increasing the efficiency of algal biohydrogen production. For example, mutant strains with increased starch reserves and inhibited cyclic electron flow around PSI (*Stm6*) and with enhanced external supply of glucose (*Stm6glc4*) have shown significant improvements [42, 95].

To develop economically viable algal H_2 production systems, the metabolite flux to H_2 will have to be further optimized through bioengineering and by optimization of process-related parameters in closed bioreactor systems [76]. Bioengineering includes approaches to improve the photon conversion efficiency rates from currently ~1% to economically profitable rates of ~7% [76]. Details on recent bioreactor design and ways to bring down the costs are summarized in "Closed Bioreactor Designs" section.

Coupling H_2 Production to Desalination Algal biohydrogen generation can also in principle be coupled to desalination, although at relatively low production rates. Marine and halophilic algae can extract hydrogen (as protons and electrons) and oxygen from sea water and upon combustion of hydrogen and oxygen, fresh water is produced. Consequently by using stationary fuel cells that use hydrogen and oxygen to feed electricity into the national grid, energy generation can be coupled with desalination. Although the fresh water yield is not large, it does provide a net fresh water gain whereas conventional crops do not. The yield of water is directly related to the yield of H_2. At 1% light to H_2 efficiency (the approximate current status at outside light levels using light dilution reactors) our feasibility study indicates that upon successful development of the process, a 1 Million litre photo-bioreactor facility could produce up to 610 m^3 (610,000 L) of fresh water per year.

Coupling H_2 Production to Carbon Sequestration Hydrogen is unique among biofuels in that it is carbon-free. Consequently during H_2 production the vast majority of the CO_2 sequestered during the aerobic phase remains in the residual biomass at the end of the process. By converting this biomass to Agri-char, H_2 fuel production can therefore be coupled to atmospheric and industrial CO_2 sequestration. As carbon trading schemes come on stream internationally, this would add considerable value to the overall bio-H_2 process.

Algal Biomethane Production

The production of biogas from biomass is gaining increasing importance worldwide. Recent studies of the Institut für Energetik und Umwelt Leipzig predict that a significant part of the methane demand in Europe can be satisfied with biogas [18]. However, the knowledge of the biological processes which take place in a biogas production facility is limited today; therefore research in this field is needed and important to improve the biomethane production process. Biomethane can be made from a very wide range of biomass crops as well as from a range of crop residues.

A major limiting factor for the future growth of biomethane production from plant sources is the availability of photosynthetically grown biomass. Currently, a 500 kW biomethane plant requires approx. 10,000–12,000 tons of biomass feedstock per year with maize currently being the major crop plant feedstock. Using cereals and sunflowers, typical yields between 2,000 to 4,500 m^3 biomethane per hectare per year have been reported [5, 192]. Yields from maize are higher and vary in dependence of the species and the time of harvesting between 5,700 and 12,400 m^3 of biomethane per year per hectare [5, 136, 192]. For certain grass species like ryegrass, yields of up to 4,000 m^3 of biomethane per year per hectare were reported [192].

Microalgae are a major focus of interest as the efficiency of biomass production per hectare is estimated to reach 5–30 times that of crop plants [162]. The relatively high lipid, starch and protein contents and the absence of lignin that can not be fermented easily, make microalgae an ideal candidate for efficient biomethane production by fermentation in biogas plants. Similar to biodiesel, lipids play an important role, since their conversion capacity into biomethane is higher (1390 L biogas (72% CH_4, 28% CO_2) per kg organic dry substance) than that of proteins (800 L biogas (60% CH_4, 40% CO_2) per kg organic dry substance) and carbohydrates (746 L biogas (50% CH_4, 50% CO_2) per kg organic dry substance) [184].

One of the first studies on the feasibility of using microalgae for biomethane production was published almost 50 years ago and concluded that the process could be feasible and could be further optimized in the future [65]. Using highly efficient closed algal bioreactors for biomass feedstock and biomethane production, microalgae can now be grown in large amounts (150–300 tons per ha per year; [29, 138]). This quantity of biomass can theoretically yield 200,000–400,000 m^3 of methane per ha per year. However, it has to be pointed out that biomethane production from microalgae currently is not competitive with biomethane production from maize or other crops because the production of biomass is expensive (in Germany, where most of the biogas production facilities are located, market prizes e.g. for the green alga *Chlorella vulgaris* are in the order of 100 times higher than the price for maize), and production capacity is far too low today to feed the demand of commercial biogas plants. Methane produced in biogas facilities today is mixed with carbon dioxide gas (usually 50–75% methane) and the high degree of impurity limits its use. Biogas combustion is usually carried out in combined heat and power plants, and therefore traditionally no storable fuels are currently produced. However, the development of efficient purification systems are already under way and have led to the construction of first pilot plants for biomethane separation in Austria in 2005 (patent no AT411332B (2003)) and in Germany in 2006 (patent no.304 26 097- BCM-method).

Cultivation of Microalgae

Conventional open pond algal production systems and even some closed algal bioreactors have already achieved economic viability in the production of high value products such as astaxanthin and nutraceuticals. As discussed in "Economic Feasibility of Microalgal Biodiesel" section, economic margins are much smaller for biofuel production systems as their market value is much lower. Optimized biomass production is therefore central to economic biofuels production [73, 76, 100, 145, 166] and this in turn requires careful optimization of cultivation systems.

Optimizing Culturing Conditions

The optimization of strain-specific cultivation conditions is of confronting complexity, with many interrelated factors that can each be limiting. These include temperature [30], mixing [12], fluid dynamics and hydrodynamic stress [13], gas bubble size and distribution [14, 135], gas exchange [52], mass transfer [68], light cycle and intensity [87, 132], water quality, pH, salinity [1, 30, 140, 141], mineral and carbon regulation/bioavailability, cell fragility [70], cell density and growth inhibition [16]. While shaking cultures under controlled conditions may present valuable preliminary data, valid scale-up models require properly engi-

neered lab-scale bioreactors [150] in order to match mixing and mass transfer conditions (see "Closed Bioreactor Designs" section).

The vast majority of research into algal growth and nutrient relations has traditionally been devoted to algae in its natural environments and the role of algal growth within ecosystems. In contrast, algal production systems require maximum biomass growth to very high cell densities. A better understanding of the physical principles and bioreactor design [66] has increased the current achievable cell density. Optimal media formulation is also critical to ensure sufficient and stable supply of nutrients to attain maximal growth acceleration and cell density, and ultimately to produce biofuels at higher efficiencies [36, 37]. Algal production can also be a multi-phased process with each step having independent optimal conditions such as nitrogen limitation in oil production [165] or sulphur limitation in H_2 production [111].

Batch feeding of heterotrophic algal cultures [100] and CO_2 enrichment of photoautotrophic algal cultures can significantly increase biomass, and optimization of mineral nutrients can increase culture productivity. Nitrogen and phosphorous are generally early targets in mineral optimization of media formulations [28, 86, 165, 203], but other minerals are also vitally important for support of the structural and metabolic biochemistry of the cell. Mineral ions also have significant impact upon areas such as osmoregulation and osmoadaptive capacity [89, 90], and molecular configuration of photosynthetic complexes [112].

Certain algal strains when grown with yeast extract, grow at twice the rate and to twice the cell density. The strains that show improved growth are clearly able to assimilate the dissolved organic molecules, and consequently in some circumstances waste water can therefore be considered a resource (see "Using Wastewater and Seawater Resources" section). The increasing scarcity of fresh water resources in many countries also makes this recycling of waste water attractive, and different aspects of waste water usage continue to be investigated [10, 153, 154, 163, 201]. Zaslavskaia et al., [202] demonstrated the conversion of *Phaeodactylum tricornutum*, an obligate photoautotroph, to a heterotroph through the biolistic transformation with a single gene encoding a glucose transporter. The identification, characterization, and engineering of transporters show great potential in future applications of algal culture in general and in particular for biofuel production (see "Metabolic Engineering and Systems Biology Approaches" section).

Maintenance of an acceptable pH range throughout culturing is of utmost importance as it impacts all aspects of media biochemistry. Both, ionic absorption from the media and the metabolic biochemistry of the cell, exert significant pressure upon pH and in high performance cultures their effect is powerful enough to overcome the neutralizing capacity of exogenous buffering agents. Currently microinjection of strong acids and alkalis, metabolic balancing in heterotrophic cultures and regulated CO_2 dissolution [52, 173] in both photoautotrophic and heterotrophic cultures, are the most practical and economical strategies for pH control.

Open Pond Systems

The vast bulk of microalgae cultivated today are grown in open ponds. Open ponds can be built and operated very economically and hence offer many advantages as long as the species for cultivation can be maintained [193]. Open ponds have a variety of shapes and sizes but the most commonly used design is the raceway pond. An area is divided into a rectangular grid, with each rectangle containing a channel in the shape of an oval; a paddle wheel is used to drive water flow continuously around the circuit. They usually operate at water depths of 15–20 cm, as at these depths biomass concentrations of 1 g dry weight per litre and productivities of 60–100 mg L^{-1} day^{-1} (i.e. 10–25 g m^{-2} day^{-1}) are possible [137]. However, such productivities are not the rule and cannot be maintained on an annual average. Similar in design are circular ponds which are commonly found in Asia and the Ukraine [15]. Algae ponds used in wastewater treatment plants are built in whatever shape best suits the location; these are usually not mechanically mixed but driven by gravity flow. One of the largest of this type of algal pond is Melbourne's Werribee wastewater treatment plant spanning 11,000 ha.

In the case of algal wastewater treatment ponds, retaining walls or dug trenches form the basis of the ponds. Raceway ponds are more expensive to construct due to the extra infrastructure required (paddle wheel) and the faster flow rates mean more stable structures are required to ensure that the pond integrity is maintained. But, because no transparent material is required in the construction of open ponds, a very broad range of materials can be used for the construction. Open ponds are also relatively easy to maintain since they have large open access to clean off the biofilm that builds up on surfaces.

The main disadvantage of open systems is that by being open to the atmosphere, they loose water by evaporation at a rate similar to land crops and are also susceptible to contamination by unwanted species. A new open pond is typically inoculated with the desired algal culture with the aim of initiating growth and dominating the pond flora. However, over time undesired species will inevitably be introduced and can severely reduce yields and even outcompete the inoculated species. Once a significant competitor has taken residence in a pond it is extremely difficult to eradicate. From the aquatic species program collection of over 3,000 photosynthetic organisms, none were found to

be able to continually dominate an open pond and have desirable biofuel properties, i.e. high lipid contents [162]. In practice open ponds are usually reported to be dominated by two to six species with a range of evolutionary advantages: rapid growth, resistance to predators, tolerance to high levels of dissolved oxygen, etc.

Sustained and reliable cultivation of a single species in open pond systems can however be encouraged by cultivating extremophiles that tolerate and outcompete other species in a particular environment (e.g. high/low pH or salinity). *Spirulina* for example survives and grows well at high pH (9 to 11.5) and is commonly the dominant species in soda lakes [17]. Because of its spiral shape it is also easy to harvest. Australia is the largest producer of *Dunaliella salina* in the world. This unicellular green algal species grows very well in highly saline waters due to its high intracellular glycerol content, which provide protection against osmotic pressure. *Dunaliella salina* is an excellent source of valuable carotenoids which protect it against the intense light it is subjected to in the shallow salt pan ponds [21].

Closed Bioreactor Designs

Besides saving water, energy and chemicals, closed bioreactors have many other advantages which are increasingly making them the reactor of choice for biofuel production, as their costs are reduced (Fig. 3). The most important among these aspects is that they support up to fivefold higher productivity with respect to reactor volume and consequently have a smaller "footprint" on a yield basis [13]. The latter point is the deciding factor, as the goal is to collect as much solar energy as possible from a given piece of land. Higher bioreactor costs are therefore compensated for by higher productivity as recent studies show (e.g. [29]). Most closed photo-bioreactors are designed as tubular reactors, plate reactors, or bubble column reactors ([137, 193]; Figs. 3 and 4). Other less common designs like semi-hollow-spheres have been reported to run successfully [152]. However, there is still a gap between designing a high-end reactor which meets all demands of the algal cells on the one hand, and a cheap reactor on the other hand, which enhances the economic viability of the process [193]. Based on the current energy costs and the given productivity, reactor costs should not exceed US $15 per m^2. In the following paragraph some aspects and current developments of reactor design are discussed in the view of economic feasibility.

Most microalgae exhibit growth/light kinetics at which light saturation occurs at modest light intensities. Consequently in direct sunlight they show low efficiencies, photo-inhibition, or even photo-bleaching (e.g. for *Chlamydomonas*; [110, 134]. To increase process efficiencies photo-bioreactor have to be designed to distribute light over a large surface area in order to provide moderate light intensities for the cells ('light dilution'). This is usually achieved by arranging tubular reactors in a fence-like construction (e.g. Fig. 3). The fences are oriented in a north/south direction to prevent direct bright light hitting the surface. In this way sunlight is "diluted" in a horizontal and vertical direction. Using such systems up to 47 g dry weight m^{-2} day^{-1} can be obtained [27]. Taking reflection into account, the light is distributed quite satisfactorily over the circumference of the tube. However, this is mostly done simply by experience and not by precise measurement and the application of kinetics in contrast to other fields of (bio-)chemical engineering. To optimize the light dilution effect, the bioreactor surface area can be up to ten times larger than the corresponding footprint area. Analogous statements can be made for bubble columns or plate photo-bioreactors mounted at a defined angle to the sun. Both design types assist the optimization of cell growth, but require more transparent surface materials like glass or plastics. Nevertheless, one basic design principle is to "make the surface to volume ratio as big as possible". Values of 400 m^2/m^3 are state of the art and such designs result in

Fig. 3 A high-end closed bioreactor system. The worlds largest closed photo-bioreactor in Klötze, near Wolfsburg, Germany (Bioprodukte Prof. Steinberg; www.algomed.de); the 700 m^3 are distributed in 500 km of tubes and produce up to 100 t algae biomass per year

Fig. 4 Flat panel airlift from Subitec [38, 102] with usage of the flashing light effect, here for astaxanthin production

shorter light path lengths and can therefore support higher biomass concentrations. As a result, for a given biomass yield a small culture volume and less energy for mixing is required.

Mixing is necessary in all photo-bioreactors as it prevents sedimentation of the cells and supports distribution of CO_2 and O_2 [117]. For CO_2 a partial pressure of at least 0.15 kPa has to be maintained to prevent kinetic CO_2 uptake limitation and a stoichiometric demand of 1.7 g CO_2 per g biomass has to be provided. That makes supply with CO_2-purified from external flue gas e.g. from a power plant useful [45]. While light attenuation inside the reactor is not influenced by mixing, there is a complex interplay between culture mixing and light attenuation as each single algal cell passes through dark and light zones of the reactor in a more or less statistical manner [13]. Dark zones appear by mutual shading of cells at the reactor side furthest removed from the incident light. Consequently, another look at the algal physiology is necessary in the context of the so called "flashing light effect" [69].

Although high light intensities load the photosystems with energy, they can also result in photoinhibition. Consequently as the downstream metabolic steps are relatively slow, micro-algae have evolved photoprotective mechanisms which dissipate (i.e. in the context of biofuel production, waste) excess energy more rapidly as fluorescence and heat. This energy wastage can be avoided, when the algal cells run through the low light/high light cycle induced by mixing as the low light phase allows the energy in the photosystems to be channelled into the downstream metabolic processes. These cycles should be in frequencies of 10 Hz or faster with the dark period being up to ten times longer than the light period [85]. The algal cells then behave similarly to being under constant moderate light exposure [199]. In photo-bioreactors the flashing light effect can be fully harnessed by intelligent mixing, the optimized transfer of cells from dark zones to bright zones and vice versa in a regular manner. This concept has been realized, for example in the reactors shown in Fig. 4 [109]. The reactor is basically a plastic plate airlift reactor with in-built baffles. These induce constant horizontal vortices of the fluid. However, the drawback here is the energy consumption for mechanical mixing. Nevertheless, these kind of reactors are successfully employed. A first demonstration plant for CO_2-fixation is going to be built in Hamburg, Germany in 2008. In many different reactors, mixing energy is calculated as being 10–30% of the incident light energy. This is clearly too much as it exceeds the net energy outcome. Sparing mechanical energy input at the lower end of the energy necessary for mass transfer and to prevent sedimentation can bring it down to 1%, (10% of the final energy turnover). This is currently only possible using thin layer reactors and regular flow patterns. New developments are under way to address this problem in other types of reactors.

In the last few years many attempts have been made to refine the above principles and incorporate these concepts into systems made of more economical materials. Plastic (polyethylene) bags mounted as annular reactors (to avoid dark zones in the inner part of the cylinder) or as plate reactors are commercially available [146, 179]. Simple plastic tubes mounted horizontally on the soil proved to be less than robust. One of the latest developments is the triangular reactor (Fig. 5). It combines the principle of a bubble column with mixing by in-built static mixers in an external 'downcomer'. According to press releases from MIT and an external evaluation [139] this "3DMS-Reactor" exhibits an average productivity of 98 g dry weight m^{-2} day^{-1} over a period of 19 days even when under sub-optimal lighting conditions. Thus, this is one of the most productive algal cultivation systems ever built, and verges on the theoretical maximum average yield of 100 g m^{-2} d^{-1} (see "Recent Advances in Microalgal Production" section). Further developments now have to be made using thin layer reactors with high inner surface areas for light distribution. These maximize the biomass concentration and minimize mixing energy [150]. Furthermore, gas transport should be possible by diffusion alone, thus avoiding energetically unfavourable bubbling.

Another bioreactor design involves collecting light by plastic Fresnel lenses and guiding it into a lump reactor by optical fibres (Wijffels 2007, personal communication). Splitting the infra-red part of the solar light beam not only reduces the problem of overheating during high solar irradiance but allows the extracted heat energy to be used to generate electricity. This can be used for mixing the culture in bioreactors which is currently the most energy-intensive step. This principle would avoid a lot of problems connected with large outdoor reactors, but the implementation cost is high and the light conduction technology has to be developed further with the aim of reducing its cost

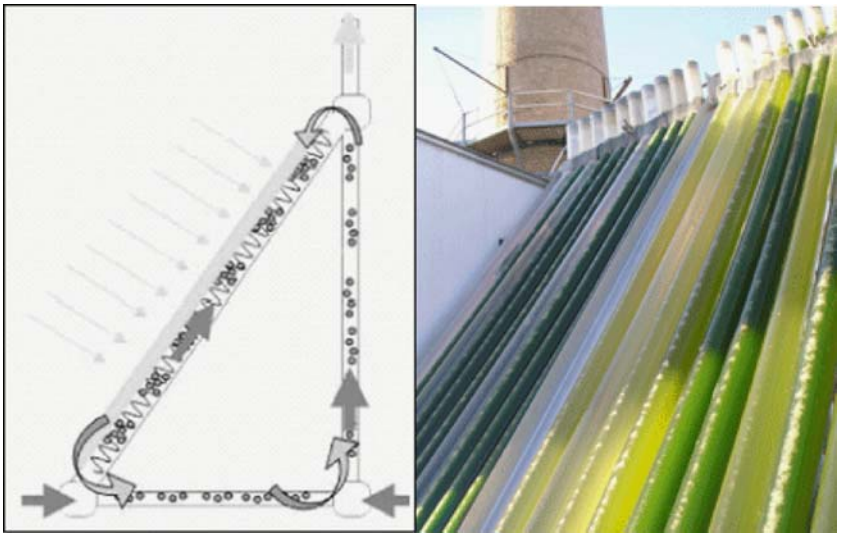

Fig. 5 GreenFuel's 3D Matrix Algae Growth Engineering Scale Unit, "triangle airlift reactor". At the left there is the drawing from patent US 20050260553, at the right the demonstration plant at the Red Hawk Power Plant, Arizona, USA [50]

and making it more efficient. A basic schematic of four commonly used bioreactor designs are presented in Fig. 6.

In summary, photo-bioreactor design especially for biofuel production, is a rapidly expanding field central to fast-tracking the development of second generation micro-algal biofuel systems. Only new design principles in connection with the knowledge and consideration of microalgal kinetics and growth dynamics can lead to optimal and economically viable systems.

Hybrid Systems

Open ponds are a very efficient and cost-effective method of cultivating algae, but they become contaminated with unwanted species very quickly. Photobioreactors are excellent for maintaining axenic cultures but setup costs are generally ten times higher than for open ponds. A combination of both systems is probably the most logical choice for cost-effective cultivation of high yielding strains for biofuels. Inoculation has always been a part of algal aquaculture. Open ponds are inoculated with a desired strain that was invariably cultivated in a bioreactor, whether it be as simple as a plastic bag or a high tech fibre optic bioreactor. Importantly, the size of the inoculum needs to be large enough for the desired species to establish in the open system before an unwanted species. Sooner or later though contaminating species will end up dominating an open system and it will have to be cleaned and re-inoculated. Therefore to minimize contamination issues, cleaning or flushing the ponds should be part of the aquaculture routine, and as such, open ponds can be considered batch cultures. This process has been demonstrated by Aquasearch (Hawaii, USA) cultivating *Haematococcus pluvialis* for the production of astaxanthin. Half of the Aquasearch facility was devoted to photobioreactors and half to open ponds. *H. pluvialis* is grown continuously in photobioreactors under nutrient sufficient conditions and then a portion is transferred to nutrient-limited open ponds to induce astaxanthin production. Enough nutrients are transferred with the inoculum for the culture to continue to grow for 1 day, and after 3 days

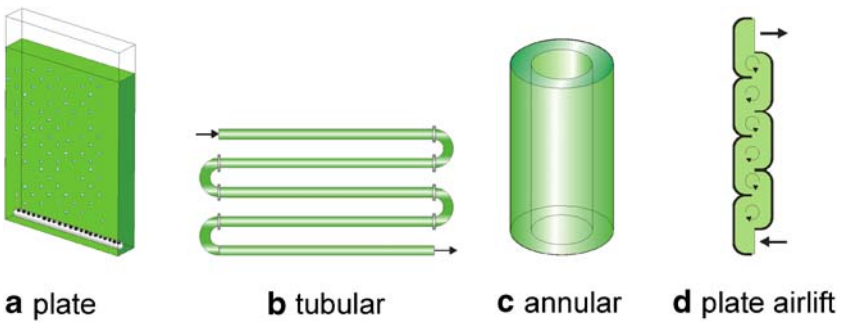

Fig. 6 Different closed photobioreactor designs commonly employed for production of valuable compounds: **a** plate reactor, the classical approach, **b** tubular reactor, biggest closed photobioreactor is made in this design, **c** annular reactor, acts as bubble column, the inner cylinder is empty to avoid dark parts and to increase surface/volume ratio, **d** plate airlift reactor with baffles supports flashing light effect by controlled fluid barrels. All these designs are regarded as in general being suitable but also being too expensive for biodiesel production

when astaxanthin level peak, the open ponds are harvested, cleaned and then re-inoculated [83]. This approach is also very suitable for biofuel production as under low-nutrient conditions algae rapidly start to convert energy from the sun into chemical energy stored as lipids as a means of survival (see "Lipid production by microalgae in nature" section).

For large scale microalgae biofuel production there would need to be a series of photobioreactors of increasing size, from starter culture through to the final inoculum. As the bioreactors increase in size, the level of complexity should be reduced to minimize the cost per square meter. Smaller bioreactors need to be kept strictly under axenic conditions but as the bioreactor size increases the level of containment can be relaxed if there is a continual resupply of inoculum to flush each stage of the scale up, provided there are protocols in place to eradicate contamination if it takes hold early in the scale up chain. For such an approach to work, it is important to use an algal species that is both, fast growing during the inoculum scale-up stage and highly productive in the final open pond stage. This process allows a continual supply of fresh inoculum into open ponds at environmentally-friendly low nutrient conditions to avoid the dominance of invading species while encouraging the continuous production of algal biofuels.

Downstream Processing

Harvesting Methods Algae typically have a high water content and downstream harvesting and processing requires its removal. There is no single best method for harvesting microalgae and reducing their water content. In existing algal aquaculture the most common harvesting processes are flocculation, microscreening and centrifugation. Most importantly, cost-effective and energy-efficient harvesting methods are required to make the whole biofuels production process economical. In this regard, strain selection is an important consideration since certain species are much easier to harvest than others. For example the cyanobacterium *Spirulina*'s long spiral shape naturally lends itself to the relatively cost- and energy-efficient microscreen harvesting method [11, 16]. Cost-effective filtration, however, is limited to filamentous or large colonial microalgae. While filtration is often applied at a laboratory scale, in large-scale applications it suffers from problems such as membrane-clogging, the formation of compressible filter cakes and in particular, from high maintenance costs.

Sedimentation and centrifugation can be described by Stokes' law, which predicts that the sedimentation velocity is proportional to the difference in density between the cell and medium on the one hand and on the square of the radius of the cells (Stokes radius) on the other hand. While for bacteria gravitational force-based methods are not easy to apply, for yeast and microalgae with diameters >5 μm and relatively thick cell walls they are feasible. Pure sedimentation - or settling as it is called in aquaculture - is employed in some algal farms, but is time- and space-consuming and is not an appropriate choice for biodiesel production. Commercial centrifuges accelerating to at least 10,000 × g enhance separation and decanting centrifuges have also been successfully employed (e.g. [195]). Currently, centrifugation is considered to be too cost- and energy-intensive for the primary harvesting of microalgae. The energy input alone has been estimated at 3,000 kWh/ton [16]. Centrifugation is however a very useful secondary harvesting method to concentrate an initial slurry (10–20 g/L) to an algal paste (100–200 g/L) and could possibly be used in combination with oil extraction.

Flocculation, the aggregation and sedimentation (or floatation) of algal biomass, is also a very common primary harvesting method used to concentrate algae [118]. In raceway or mixing ponds, adjacent settling ponds are used for flocculation, settling and harvesting. Inorganic chemicals such as alum, ferric chloride and lime are very effective flocculants but are considered to be too expensive for large-scale operations and the algal chemical sludge can not be used for some downstream applications, such as animal feed supply or for anaerobic digestion. Organic cationic polyelectrolyte flocculants (e.g. cationic polymer Chitosan) are a preferred option as much less is required and the algae can be used in downstream processes [118]. Flocculation increases particle size and leads therefore to faster sedimentation (see Stokes' law) or better, interaction with floatation bubbles. Nevertheless, addition of flocculants is currently not a method of choice for cheap and sustainable production. Recent developments involve encouraging self-flocculation of the cells, which can occur during carbon limitation or pH shifts.

Polyelectrolytes can also be used to assist spontaneous flocculation (bioflocculation). Bioflocculation is likely the cheapest harvesting process. Certain species naturally flocculate, while others flocculate in response to environmental stimuli, nitrogen stress, pH and level of dissolved oxygen [16]. The problem however with spontaneous flocculation is the time it can take to occur which is not always reliable. An interesting variation of this, recently presented by Ami Ben-Amotz, is co-bio-flocculation [3]. Here the naturally flocculating alga *Skeletonema* was used to form flocs with high lipid varieties of *Nannochloropsis*. Another interesting method recently presented by Mike Massingill, is to feed the algae to the fish Tilapia (*O. mosambicus*) which obtain very little nutrient from it. The algal biomass is then harvested from the sedimented droppings by a conveyor belt (10–14% solids) and then air-dried ([3]; Kent Seatech: United States Patent 6447681).

Lipid Extraction and Transesterification The majority of biodiesel today is produced from animal or plant oils through a transesterification process following oil extraction with or

without cell disruption. Alternatively the process can be facilitated by combining the use of immobilized lipases with methyl esterification [100]. In essence, a common extraction process involves mechanical crushing followed by squeezing. While cell disruption can be carried out by high-pressure homogenization ("French press") a modern approach is electroporation, where a high electrical field is applied to the biomass leading to perforation of the cell wall and to better extraction. For extraction of oils and other microalgal products, chemical solvents can be chosen in one- or two-step extraction approaches. This can even be applied to living algae in situ [79] or combined with transesterification using methanol and a catalyst such as sodium methoxide to produce biodiesel and glycerol [58].

CO_2-Sequestration

Atmospheric CO_2 levels have already exceeded 450 ppm CO_2-e and are at levels classified as "dangerously high" [84, 170]. So although the development of CO_2-neutral biofuel production systems is important, their production will largely serve to stabilize atmospheric CO_2 levels at a "dangerously high" level (an important first step), rather than actively reducing it back down to an acceptable concentration. Physical sequestration of atmospheric CO_2 is often considered challenging as it is technically difficult to separate CO_2 from other atmospheric gases. Photosynthetic organisms have however fine-tuned this process over millions of years and of course are well adapted to capturing CO_2 and storing it as biomass. If this captured CO_2 could therefore be converted to a more stable form for long term storage (~100 years or more) it would open up the important opportunity to couple CO_2-neutral biofuel production (e.g. biodiesel) with atmospheric CO_2 sequestration.

Weissman and Tillett [194] studied the capture of carbon dioxide by large pond-type systems. When operating under optimum conditions, the capture efficiency has been shown to be as high as 99% [194, 204]. Based on the following equation, 1.57 g of CO_2 is required to produce of 1 g of glucose:

$$6CO_2 + 12\ H_2O + \text{light} + \text{chlorophyll} = C_6H_{12}O_6 + 6\ O_2 + 6H_2O$$

Kurano et al. [97] reported fixation of 4 g CO_2 L^{-1} day^{-1} at growth rates of 2.5 g algae L^{-1} day^{-1}, a ratio of 1.6 to 1. Taking into account the conversion of glucose into other compounds such as lipids or starch under certain conditions; the consumption of CO_2 can be as high as 2 g CO_2 to 1 g algae. Assuming a growth rate of 50 g m^{-2} day^{-1} it is possible for one hectare of algal ponds to sequester up to one ton of CO_2 a day.

In the case of the biodiesel process, after extraction of oil (e.g. ~30% of the dry weight), the remaining 70% biomass can be fed into downstream carbon sequestration processes. Specifically the sequestered carbon can be converted to hard C-chips (Agri-char) via the 'slow burning' process of pyrolysis [25]. This has the additional advantage that Agri-char, as its name suggest, can be marketed to the agricultural sector, as it greatly enhances the carbon content of the soil and so its fertility. This in turn can contribute further to climate change mitigation by affecting the gas exchange of crops and soils [98, 105]. Furthermore, pyrolysis acts as a sterilization process of the biomass waste, providing an environmentally-sensitive waste disposal mechanism that will increasing the public acceptance of the use of genetically modified microalgae for biofuel production.

Molecular Improvements of Microalgae for Increased Biodiesel Yields

To obtain the best performing microalgae strains for biofuel production, one can (1) screen a wide range of natural isolates, (2) improve them by metabolic (genetic) engineering or (3) by selection and adaptation. Algae collections worldwide contain thousands of different algal strains that can be accessed, for example information about available cultures of algae strains in Europe can be obtained via the Algi-Net Database [4]. Notably, the US Aquatic Species Program had collected 3000 algal strains and assessed these for potentials of biofuel production [162]. Global algae collections and species maintained from the Aquatic Species Program combined with recent advances in genetic engineering and material sciences, provide a good starting point for further development of microalgal biodiesel production systems. Future algal strain improvement will utilize methodologies such as lipidomics, genomics, proteomics, and metabolomics to screen for and develop new strains that exhibit high growth and lipid biosynthesis rates, broad environmental tolerances, and that produce high value-add by-products.

Metabolic Engineering and Systems Biology Approaches

Bottle necks in algal biodiesel production within the cell can be identified by transcriptomics, proteomics and metabolomics/metabonomics approaches. In contrast to a pure genomics approach the integrated use of these methods allows insight into cellular processes. The identification of differentially expressed genes, proteins or metabolites gives clues to rate-limiting processes in the cell, which can be backed up by the determination of metabolic flux. This systems biology approach will allow fine-tuning of algal properties by genetic or metabolic engineering.

Metabolomics aims to determine metabolic profiles to define the metabolome of a given organism [56], in this case lipid-rich algae. Metabonomics, the statistical identification of differences in metabolite levels due to genetic or environmental changes using NMR spectroscopy or mass spectrometry (in combination with various chromatography techniques) followed by chemometric analysis [101, 123, 124], allows determination of accumulated metabolic end products and intermediates. The interpretation of these results alone is not straightforward as the accumulation can be due to an up-regulated enzyme downstream, or a down-regulated enzyme upstream, in the metabolic pathway. Therefore this data should be interpreted in context with transcriptomics and proteomics results (e.g. [121]). To resolve the metabolic dynamics of microalgae, the metabolic flux can be studied by various techniques such as the monitoring of consumption and production of key compounds [44, 197], or the isotopic labelling of key metabolite precursors or intermediates and the monitoring of these isotopes in a time-dependent manner [55]. Radioactive isotopes (e.g. [34, 158, 198]) or stable isotopes for NMR spectroscopy (e.g. [159, 169]) and mass spectrometry (e.g. [158, 161, 185, 198]) can be used to obtain a time- and in some cases spatially-resolved picture of the metabolic flux of an isotope from a given starting compound to a metabolic end product.

Transcriptomics and proteomics offer the additional possibility of identifying differentially expressed genes and proteins that are either directly involved in lipid biosynthesis and degradation or that are coordinately regulated. For example, the identification of key regulatory genes and their proteins, such as transcription factors, kinases and phosphatases, and their over- or under-expression in transgenic cells can efficiently alter whole physiological pathways (e.g. [6, 43, 107]. Fatty acid production and composition has been altered in a number of plants by metabolic engineering using transgenes encoding for different enzymatic steps in fatty acid biosynthesis/modification pathways, most notably in canola (e.g. [39, 49]).

After identification of the pathways and key enzymes involved, genetic engineering has the potential to improve algal productivity. Routine transformation is currently carried out only for a few selected algal model species including *C. reinhardtii*, however, the growing field of transgenic microalgae has considerable potential [99, 189]. Different transformation methods are available for the delivery of DNA into the algal genome with the 'biolistic' technique being the most common one [189]. This technique involves bombardment with DNA-coated microprojectiles and has been successfully used for a variety of algae including green algae and diatoms [8, 94], and is also the method of choice for chloroplast or mitochondrial genome transformation [143]. Other methods to create transgenic algae are the agitation of cells in the presence of glass beads and DNA [88], agitation with silicon-carbide whiskers [46, 47], and electroporation [164, 174]. More recent developments for the improved overexpression of transgenes, involve the use of vectors containing nuclear matrix attachment regions (MARs) to increase the expression level of foreign genes. This has been carried out in the halotolerant algae *Dunaliella salina* [191].

Using the glass bead technique of Kindle [88], Doebbe et al. [42] were able to introduce HUP1 (hexose uptake protein) hexose symporter from *Chlorella kessleri* into the *C. reinhardtii* mutant *Stm6.* This resulted in an engineered mutant that can use externally supplied glucose for hydrogen production. Another starting point for the metabolic and genetic engineering of microalgae is the engineering of the photosynthetic light capture machinery in order to improve solar energy to biomass conversion ([122]: see Photosynthetic Efficiency section).

The genome of *C. reinhardtii* has recently been sequenced and revealed among others, previously unknown genes associated with photosynthetic functions [113]. Electron pathway optimization can also be considered with the aim of increasing PQ levels by genetically modifying parts of the PQ pathway to decrease photodamage and increase photosynthetic efficiency under high light conditions, and therefore, to increase the biomass production rate [122].

The biosynthesis of algal lipids requires acetyl-CoA as the starting point. Acetyl CoA carboxylase and other enzymes of the lipid biosynthesis pathway have been used as targets for improving oil production [142, 162]. Lipid metabolism, and the biosynthesis of fatty acids, glycerolipids, sterols, hydrocarbons and ether lipids in eukaryotic algae have been recently reviewed in the context of optimization for biodiesel production [72, 115]. While *C. reinhardtii* serves as a model organism to study lipid biosynthesis in green algae [205], some unusual hydrocarbons and ether lipids from *Botryococcus braunii* have been described (e.g. n-alkadienes, trienes, triterpenoid botryococcenes, methylated squalenes, tetraterpenoids; lycopadiene; [2, 115].

The type of oil used as a biodiesel feedstock has a large impact on the quality of the fuel product. Genetic engineering of key enzymes in specific fatty acid production pathways within lipid biosynthesis is therefore a promising target for the improvement of both quantity and quality (chain length and saturation grade) of lipids.

Lipid quality is an important issue for biodiesel production, as the alkyl ester content dictates the stability and performance of the fuel, and this in the end is an important factor in meeting international fuel standards. The volumetric energy density of biodiesel is about 33 MJ/L, which

is about 92% of petrodiesel and so essentially comparable. However, the average hydrocarbon length is longer in biodiesel, resulting in combustion that is hotter and sustained for longer than in conventional diesel fuel. A more complete burn of the fuel is achieved. When taking this into consideration the overall efficiency of biodiesel is approximately 97% of that of petrodiesel [92].

Biodiesel is currently produced from a number of different oilseed crops, most commonly soybean, rapeseed and palm oil. As the triacyl glyceride (TAG) profile of a given biofuel crop is relatively consistent, the property of biodiesel produced from a particular crop has predictable qualities. There are a number of factors that must be considered when developing a new source of TAGs into biodiesel. Microalgal lipids are predominantly polyunsaturated, and so are more prone to oxidation. This is a serious issue with biodiesel whilst in storage. This drawback, however, can be corrected through partial catalytic hydrogenation of the oil [29]. Gunstone and Hilditch [71] measured the relative rate of oxidation for the methyl esters of oleic (18:1), linoleic (18:2), and linolenic (18:3) acids to be 1:12:25. It is therefore preferable that the level of polyunsaturated fatty acids in biodiesel is kept to a minimum. In contrast, higher levels of polyunsaturated fats lower the cold filter plugging point (CFPP); the temperature at which the fuel starts to form crystals/solidifies and blocks the fuel filters of an engine. Table 2 presents the melting point of the major fatty acids. It can be seen that the more unsaturated an oil is, the lower the melting point. Therefore colder climates require a higher unsaturated lipid content to enable the fuel to perform at low temperatures. Cetane number (Table 2) is another measure describing the combustion quality of diesel fuel during compression ignition. In a particular diesel engine, higher cetane fuels have shorter ignition delay periods than lower cetane fuels. Therefore it is important to ensure that the cetane number of biodiesel meets the engine cetane rating [91]. With these considerations in mind, the "ideal mix" of fatty acids has been suggested to be 16:1, 18:1 and 14:0 in the ratio 5:4:1 (William Klopfenstein, personal communication). Such a biodiesel would have the properties of very low oxidative potential whilst retaining a good CFPP rating and cetane number.

Algae have excellent potential for the genetic modification of their lipid pathways; e.g. by up-regulation of fatty acid biosynthesis or by downregulation of β-oxidation. By knocking out or modifying enzymes responsible for the synthesis of polyunsaturated lipids in the cell, it should be possible to dramatically increase the proportion of monounsaturated lipids. Additionally, it is likely that the algal cells homeostasis mechanism would have to modify the lipid ratio to remain fluid at low temperatures by decreasing its levels of saturated lipids. The lipid profile of an algal species will remain consistent provided it is grown under the same conditions. However every algal species will have its own lipid profile and it is therefore important to utilize species that have a suitable lipid profile for biodiesel production.

Photosynthetic Efficiency

Apart from metabolic engineering approaches to increase lipid production, two other examples are briefly described below that have potential usefulness for large-scale cultivation: the increase of photosynthetic efficiency, as well as the selection and improvement of strains for optimal growth, survivability, and oil production using wastewater and seawater resources.

Any increase in photosynthetic efficiency will enhance downstream biofuel production. Photosynthesis drives the first stage of all biofuel production processes (Fig. 1). Specifically, it captures solar energy and stores it as chemical energy (e.g. oil, starch). Consequently increasing the light capture efficiency is a significant innovation in the development of all second generation biofuel production systems. Most wild-type microalgae have evolved genetic strategies to assemble large light-harvesting antenna com-

Table 2 Profiles of fatty acids [92]

Fatty acid	Fatty acid	Cetane N°	Melting point (°C)	Ester m.p.
8:00	Caprylic	33.6	16.7	Ethyl, −43°C
10:00	Capric	47.7	31.6	Ethyl, −20°C
12:00	Lauric	61.4	44.2	Ethyl, −1.8°C
14:00	Myristic	66.2	54.4	Ethyl, 12.3°C
16:00	Palmitic	74.5	62.9	Ethyl, 24°C
16:1ω7	Palmitoleic	45	−0.1	NA
18:00	Stearic	86.9	69.6	Methyl, 39°C
18:1ω9	Oleic	55	14	Methyl, −20°C
18:2ω6	Linoleic	36	−5	Methyl, −35°C
18:3ω3	Linolenic	28	−11	Methyl, −57°C
20:1ω9	Gadoleic	82	23	NA
20:4ω6	Arachidonic	NA	−50	NA

plexes which capture sunlight and transfer the derived energy to PSI and PSII to drive the photosynthetic reactions (Fig. 1). In nature, the advantage of this strategy is that it maximizes light capture under low light conditions. However, the downside is that as excess light damages the photosynthetic machinery, higher plants and algae had to evolve photo-protective mechanisms [82, 130]. These typically dissipate (i.e. in the context of biofuel production, 'waste') most of the captured energy as fluorescence and heat [134]. This energy dissipation takes place largely in the light harvesting complexes associated with PSII (i.e. LHCII in Fig. 1).

It has been demonstrated that the overall light conversion efficiency of bioreactors can be markedly improved by reducing the number of the chlorophyll-binding LHC proteins in each cell [122, 134]. This strategy can be used to carefully fine-tune and optimize light capture efficiency of the antenna systems specifically for oil production. Normal wild-type algae have large chlorophyll-binding LHCII antenna systems and consequently the culture is dark green. Cell lines with small LHCII antenna systems yield cultures which are a much lighter green at the same cell density (Fig. 7a). In the wild-type case, algal cells at the illuminated surface of the bioreactor that are exposed to high light levels capture the bulk of the light, but waste up to ~90% of the energy as fluorescence and heat [122, 134]. As a result the wild-type cells located deeper in the culture are exposed to ever decreasing levels of light the further they are from the illuminated surface (see "Open Pond Systems" section). These shaded cells are prevented from capturing enough solar energy to drive photosynthesis efficiently. This in turn drastically reduces the efficiency of the overall culture.

In contrast, small antenna cell lines with reduced LHCII levels have the advantage that they improve the light penetration into the bioreactor (Fig. 7a) and better match it to the energy requirements of each photosynthesizing cell. Thus 'small antenna' cells at the bioreactor surface absorb only the light that they need, largely eliminating fluorescence of excess energy. This in turn allows more light (i.e. the light wasted in wild-type as fluorescence and heat) to penetrate into the bioreactor so that even cells deeper in the culture have a near optimal exposure to light [122]. Overall, therefore small antenna cultures have a higher photosynthetic efficiency (Fig. 7b). In summary small antenna mutants could offer the following advantages for biofuel production: (1) Reduced fluorescence and LHCII dependent heat losses, (2) improved light penetration properties of the culture, (3) reduced photodamage, (4) improved bioreactor efficiency and higher yield. The use of genetic engineered strains in conjunction with optimized mixing protocols ("Closed Bioreactor Designs" section) may further enhance these efficiency gains.

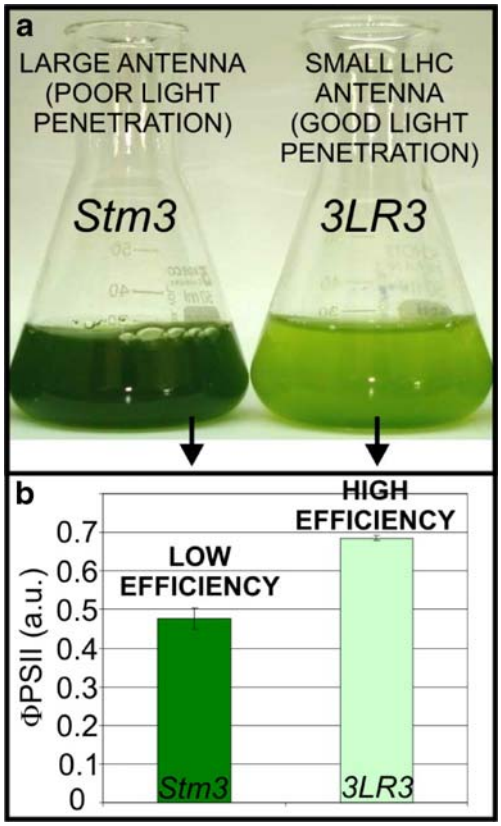

Fig. 7 Comparison of cultures of *Chlamydomonas reinhardtii* with parent strain (*Stm3*) and reduced antenna size (*3LR3*) at equal cell densities. **a** Cultures at densities of 6×106 cells/mL; **b** Photosynthetic quantum yield (φPSII); adapted from Mussgnug et al. [122]

Using Wastewater and Seawater Resources

The use of wastewater and seawater offers clear advantages over placing increased pressure on freshwater resources (as water scarcity in the face of climate change and population growth is an increasingly important issue). However, both can vary in water quality, with wastewater varying dramatically from one source to another and also fluctuating over time. Wastewater can contain valuable nutrients such as nitrogen and phosphorous [10, 163], but can also contain heavy metals, excessive trace metals, and other contaminants which are all of considerable concern—particularly in the context of biodiesel production, as heavy metals like cadmium have been reported to down-regulate lipid biosynthesis amongst many other cellular processes [64]. Seawater can also contain the same contaminants although rarely in the same concentrations. Furthermore, the use of cheaper agricultural grade fertilizers is economically desirable, but this represents another source of heavy metal contamination that can be inhibitory for sensitive strains of algae.

While conservation of freshwater resources is desirable, the selective advantage of strains that tolerate higher levels of certain contaminants can be of benefit in open pond

systems and could theoretically help to address concerns of contamination by other opportunistic organisms. Significant advances have been made towards overcoming the problem of chemical contaminations, in some part due to the use of algae in phytoremediation and toxicology studies [206] which has also been the subject of substantial debate [15, 207]. Induction of oxidative stress is considered to be the underlying problem of many metal-related contamination issues [133, 171]. As well as heavy metals [62, 186–188, 197], trace metals like copper that are normally required for nutritional sufficiency, can also be inhibitory or lethal at excessive levels [22, 180], as can other non-metal organo-compounds [54]. The mechanisms of resistance are not only dependent upon algal species but also strains within particular species and obviously upon the type of toxin. While early studies [32, 33, 108] did not fully explain the resistance mechanisms, such as reduced accumulation, sequestration, and precipitation, more recent work has made significant progress in determination of potential protein [64] and gene targets [40] involved in heavy metal resistance in algae. Structural factors such as cell wall composition [61] and parallel studies on the resistance mechanisms in plant models [116] present areas for further research.

While many algal strains flagged for oil production are marine strains, others such as *Botryococcus braunii* are freshwater strains. Thus the use of seawater for algal production can present osmotic problems in addition to those discussed above. Osmoadaptation in microorganisms has been extensively reviewed [60, 90]. Although salt stress has been shown to play a role in oil production [140, 176, 182, 183], freshwater species can undergo a stress response at osmolarities well below those of seawater, which can be strongly inhibitory or lethal. There have also been recent advances in this field with genes that exhibit anti-salt activity in microalgae [177]. Often these genes are generic anti-stress genes that likely act through alleviation of oxidative stress. A glutathione peroxidase-like protein from *Chlamydomonas* as reported by Yoshimura et al. [200] is one such example, which when cloned into tobacco induced an enhancement of stress tolerance including salt. Glutathione peroxidase has also been previously implicated in stress responses to heavy metal contamination [133].

Clearly strain selection and characterization in this context bear significant potential as well as the breeding, engineering, and adaptation of strains with desirable phenotypes that allow the use of water resources of varied water quality.

Recent Advances in Microalgal Production

Recent advances were presented at the 1st International Algae Biomass Summit in San Francisco in November [3] which brought together the heads of newly-formed microalgae biofuel companies and existing aquaculture companies, many of the chief investigators from the Aquatic Species Program and microalgae scientists from around the world [3]. World experts were invited to collaborate and apply for significant funding opportunities in 2008 and beyond. The main aim was to develop a highly-efficient system for low-cost algal oil production and to optimize conversion to JP-8. The economic targets were given as algae hydrocarbon costs of US $0.48 per litre ($2 per gallon) with a minimum order of 210 million litres (50 million gallons). At this price, algae by-products would have to factor into the economics to make it viable. The invited speakers presented a combination of lessons from history, current technology and new ideas. Key findings and conclusions can be summarized as follows: Joseph C. Weissman calculated that the theoretical maximum possible yield for algal productions is 100 g m^{-2} day^{-1} or 365 tons dry biomass per hectare per year. Current limitations include that CO_2 is not free at the high concentrations that are required for peak algal growth and that algal grazers are a significant, but a relatively ignored problem. Cost-effective harvesting has been and still is a major limiting factor. Biofuel production also reportedly requires biomass at a cost of less than $300 US/ton dry weight. Ami Ben-Amotz presented open pond yields averaging 20 g m^{-2} day^{-1} and that overall production costs of $0.34 US/kg are viable if the lipid content is high enough. A very cost-effective way for bioflocculation is the use of *Skeletonema* to co-bioflocculate high lipid *Nannochloropsis*. Tryg Lundquist reported that wastewater treatment ponds have a huge production potential and high nutrient contents and infrastructure are already in place. Limiting factors are the use of CO_2 and harvesting. Mike Massingill demonstrated that harvesting of algae assisted by fish is very cost-effective. Mark Huntley presented hybrid systems growth rates of *Tetraselmis suecica* of 62 g m^{-2} day^{-1} with 30% lipid content, although not over a yearly average. Cheap bioreactors designs were presented by Bryan Willson using disposable plastic materials and Ben Cloud at a cost ~$15 US m^{-2} that have standard farm-style set ups.

Conclusion

Global atmospheric CO_2 increases and depletion of mineral oil reserves require the rapid development of carbon-neutral renewable alternatives. Biodiesel production from micro-algae provides technical and economic feasibility that also has the potential for CO_2 sequestration and is therefore likely to find wide acceptance. Algal biofuels appear to be the only current renewable source that could meet the global demand for transport fuels. Microalgal biofuels are also likely to have

much lower impacts on the environment and the world's food supply than conventional biofuel-producing crops. The main reasons for this are high yields, a near-continuous harvest stream, and the potential to site the algal bioreactors on non-arable land. The biggest challenge over the next few years in the biodiesel field will be to reduce costs for cultivation and to further improve the biology of oil production. New materials and designs for cultivation in closed bioreactors and the use of cutting-edge metabolic engineering and screening/selection techniques are thought to provide the biggest promises.

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