Chapter 14
Algae as a Potential Source of Biokerosene and Diesel – Opportunities and Challenges

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Abstract In times of dwindling petroleum reserves, microalgae may pose an alternate energy resource. Their growth is vast under favorable conditions. However, producing microalgae for energy in an economically as well as ecologically feasible way is a difficult task and the prospects are challenging. The chapter gives an insight into perspectives of growing microalgae as a crop, highlighting some of their exceptional energy storage properties in regard to commercial exploitation. Large scale algae production techniques and concepts up to downstream processes are presented. Today, conversion to fuels is constrained by energy usage and costs – but future combination of fuel production with added value products may improve balances and lower the industrial CO_2 footprint. These challenges drive research and industry worldwide to constant improvement, supported by numerous funding opportunities. Microalgae in their tremendous diversity are a young and still very much unexplored crop. It is a challenge worth addressing.

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14.1 Introduction

The finite supply of crude oil reserves necessitates a search for alternate energy sources. Since microorganisms fix carbon photosynthetically as energy storage since 2.5 billion years [1–3], the exploitation of mass-cultivable microalgae as a possible new generation biofuel is compelling. The challenge is to fuse biology with technology – producing an economically competitive fuel without harming the environment and compromising food safety. As yet, this task only becomes economically feasible if synergistic technologies are integrated with biofuel production.

Algae, and in particular microalgae, are a generic reference to a large and diverse group of organisms that are capable of oxygenic photosynthesis. This group comprises the eukaryotic protist or multicellular green algae and the prokaryotic Cyanobacteria – not related, but commonly referred to as blue-green algae. Currently economically produced algae include eukaryotic microalgae, e.g. *Chlorella, Dunaliella, Phaeodactylum* or *Nannochloropsis,* as well as *Spirulina* (*Arthrospira*) a cyanobacterium; and also the large multicellular forms such as giant kelp (Phaeophyceae). Here the focus is put on micro-eukaryotic algae, of which many are capable of doubling their biomass more than twice a day. Microalgae could challenge conventional crops in productivity, given the right circumstances, while not being restricted to arable land [4, 5].

The fundamental ability that algae, as well as plants, use to produce energy-rich biomass is oxygenic photosynthesis. Solar light energy is used to assimilate CO_2 into carbohydrates while releasing O_2 derived from water. The subsequent cell energy storage processes provide two different products, i.e. lipids and carbohydrates (Triacylglycerols (TAG) and starch/cellulose). The first one can be directly converted into liquid hydrocarbon fuels (e.g. diesel, kerosene) while the latter requires more extensive possessing. Algal lipids as a raw material for biofuel production are an alternative to fossil fuels [6–8]. A selection of current larger-than-laboratory scale projects are listed in Table 14.1. In addition, algae synthetize numerous essential biochemical molecules, and applications of economic interest also include the production of fine chemicals, pigments, cosmetics, food or pharmaceutical additives, algae use in bioremediation/waste water treatment or CO_2 sequestration in flue gas treatment.

14.2 Algae as an Energy Source

Lipids in general, therefore also microalgal lipids, constitute a reservoir of chemical energy, a role that is of crucial importance for production of biofuels [9, 10]. It includes numerous valuable biochemical molecules. They are a very heterogeneous group of hydrophobic molecules, synthesized by several biochemical pathways and serving multiple physiological roles [11]. Lipids can be categorized in terpenes, containing pigments – carotinoids, e.g. Astaxanthin, a popular antioxidant and "salmon red"; prenylquinones, e.g. vitamin E and coenzymes; and further terpenes, also the primary constituents of essential oils, and many more of commercial interest. Terpenes e.g. from *Botryococcus braunii* are considered as potential candidates for biofuels from algae [12].

Table 14.1 Examples of currently employed or recently researched bioreactors, their use, and other microalgae-derived products

Organism	Cultivation type* and system (P=phototroph, H=heterotroph)		Produced/ calculated	Area/volume	Geographical location	Harvest type	Designated product	Productivity	NER	Cost	Reference/ manufacturer
Chlorella protothecoides	H	Fermenter	p	5–10,000 L			Biodiesel	12.8–15.5 g/(L d), 44–49 % oil		2.4 US-$/L oil	[13]
Proprietary	H	Fermenter	p/c		US, Brasil, etc.		Biofuel	70,000 t jet fuel planned from 2014 on, 42,000 t oil/a		Claim: 0.9 US-$/L oil	Solazyme
N.a.	P/H	Pond, PBR and fermenter	c		BC, Canada		Biofuel	62.5, 61.2 g/(m² d) and 100 g/L with 16–27.3 % oil content		14.44, 24.6 and 2.58 US-$/L oil	[14]
Chlorella sorokiniana	P/H	bottles	p		Spain	Centrifugation	C-N-P balance evaluation	147 (photo)-165(hetero) g/(m³ d)			[15]
Scenedesmus almeriensis	P	Tubular	p	30 m³	Spain	Centrifugation/freeze drying	Biomass	3,8 t/a		69 €/kg biomass	[16]
Proprietary	P	Vertical plastic bags	p/c		US, Florida		Biofuels Bioethanol	8–9,000 gal crude/(acre a) 2017: est. 18 million gal/a		Est. 1.30 US-$/gal (2014) refocus 2015 to CO_2 sequestration and freshwater production	Algenol

Table 14.1 (Continued)

Organism	Cultivation type* and system (P=phototroph, H=heterotroph)		Produced/ calculated	Area/volume	Geographical location	Harvest type	Designated product	Productivity	NER	Cost	Reference/ manufacturer
Nannochloropsis sp.	P	PE bag PBR	c	315 ha	US		Biodiesel	25 g/(m² d)	0.93		[17]
Scenedesmus sp., Chlorella sp.	P	Mesh Ultra-Thin Layer (MUTL)	p	500 m² 2–4 m³	Germany	Centrifugation	Biomass	2–15 g/l			[18] IGV GmbH
Haematococcus pluvialis, N. spp., C. vulgaris, P. tricornutum etc.	P	Tubular PBR	p	4 ha 85 m³	Germany	Membrane separation	Cosmetics, Omega3 fatty acids, carotenoids				Astaxa
Various	P	PBR and pond	c		–			1535 and 0.117 g/(L d), 30 % oil		1.62 and 2.08 US-$/L oil	[6]
Nannochloropsis sp., Chlorella sp.	P	Vertical plastic bags		500 m²	Germany, China	Centrifugation/ membrane filtration	Biomass	65–80 t biomass/ha 20,000 L oil/ha			[19] Phytolutions GmbH
Nannochloropsis sp., Chlorella sp.	P	Raceway pond	c	4050 ha	US	HTL	Biofuel	14.6 g/(m² d) 4 million gal/a naphta, 27 million gal/a diesel	0.41	9.8–12.4 US-$/L diesel	[20]

Table 14.1 (Continued)

Organism	Cultivation type* and system (P=phototroph, H=heterotroph)		Produced/ calculated	Area/volume	Geographical location	Harvest type	Designated product	Productivity	NER	Cost	Reference/ manufacturer
Chlorella v.	P	Airlift/acrylic glass columns	p		China	Centrifugation/freeze drying	Lipids	0.89–0.28 g biomass/(L d), 147 mg/(L d) lipids	0.38–1.25	Calculated ~63 US-$/bbl	[21]
Scenedesmus obliquus & Clostridium butyricum	P	raceway pond	p	48 m²	Lisbon	Decantation/ centrifugation	H2	7,3 g H2/kg biomass	71–100 MJ/ MJ_{prod}		[22]
Nannochloropsis sp.	P	10 l polyethylene bags	p		Lisbon	Centrifugation, drying (70°C), soxhlet, SFE	H2/lipids	0,4 g oil/g dw	127–245 MJ/MJ_{prod}	~365 €/kg oil	[23]
Nannochloropsis sp.	P	raceway pond	c	25,988 m²			Biofuel	100 t/a 0.35 g/L at 29,6 % oil content	Oil: 3.05, biomass: 8.34	0.22 US-$/kg biomass	[24]
Nannochloropsis sp.	P	Flat plate	c	10,147 m²			Biofuel	100 t/a 2.7 g/L at 29,6 % oil content	Oil: 1.65, biomass: 4.51	0.4 US-$/kg biomass	
Nannochloropsis sp.	P	Tubular horizontal	c	10,763 m²			Biofuel	100 t/a 1.02 g/L at 29,6 % oil content	Oil: 0.07, biomass: 0.2	9.5 US-$/kg biomass	

Table 14.1 (Continued)

Organism	Cultivation type* and system (P=phototroph, H=heterotroph)		Produced/ calculated	Area/volume	Geographical location	Harvest type	Designated product	Productivity	NER	Cost	Reference/ manufacturer
N.a.	P	raceway pond	c				Biodiesel	10–50 g/(m²a) 15–50 %DW lipid		750–7500 US-$/t diesel	[25]
Haematococcus pluvialis	P	Raceway pond	c		US	Gravitational, microfiltering	Methyl esters & biogas	1 kg ME & 2.6m³ biogas	0.4–0.5		[26]
N.a.	P	Raceway pond	c	4,875 ha	New Mexico, US	Flocculation, centrifugation	Algal oil	15 g/m² d^{-1} at 25% oil content 1,000bbl/d	2.73	4.10$/l oil (10y return)	[27]
Tetraselmis suecica	P	Tubular vertical	p	1 ha	Italy	Centrifuge, drying	Bbiofuel	15 g/(m² d), 36 t/(ha a)	0.59, incl. photovoltaic 1.7		[28]
Chlorella sp.	P	Airlift/glass tubes	p	100 L	China	Centrifugation drying	Biomass	0,21 g/(L d)			[29]
Chlorella v., Scenedesmus	P	V-bags	p	500 m²	Germany	Centrifugation	Biomass	0.5–1.5 g/(L d)			NOVAgreen GmbH
Chlorella v., Scenedesmus	P	Flat-panel vertical	p	200 m²	Germany	Centrifugation	Biomass/ biogas	15 g/(m² d) 900 kg/a 361 L oil/a			[30]

Table 14.1 (Continued)

Organism	Cultivation type* and system (P=phototroph, H=heterotroph)		Produced/ calculated	Area/volume	Geographical location	Harvest type	Designated product	Productivity	NER	Cost	Reference/ manufacturer
Arthrospira pratensis	P	raceway ponds	p	36,567 m²	Hawaii, US	Ocean chill drying	Biomass/ protein	>350 t biomass/a			Cyanotech
Spirulina	P	Raceway ponds	p		China		Biomass/ protein	>350 t biomass/a			Hainan DIC microalgae
Arthrospira p.	P	Raceway ponds	p	180,000 m²	CA, US		Biomass/ protein	>500 t biomass/a			Earthrise Nutritionals LLC 2012
Dunaliella sp.	P	Ponds	p	10 ha	Japan	Spray drying	Biomass/ beta carotene	2 g/(m² d), 70 t/a		17 US-$/kg DW	Nature Beta Technologies
Haematococcus p.	P	Tubular	p	300 km tubes, 4 ha	Israel	SC CO_2 extraction	Astaxanthine				ALGATechnologies
Dunaliella sp.	P	Ponds	p	250 ha, 106 m³	Hutt Lagoon, Australia		Biomass/ beta carotene	0,1–0,5 g DW/L			Cognis [31]
Proprietary	P	Raceway ponds	p	40 ha (120 ha)	New Mexico, US		Omega oils				Sapphire Energy
Proprietary	H	Fermenter	p	–	France		Omega oils	>2 t DW, 1 t oil			Fermentalg
Variable	P	floating bags (on sea)	p	0.5–12.5 m³ per PBR	Bangladesh/ CAUS	Electro water separation	Biomass/ biofuels	25 t DW/ month			Algasol/Origin Oil (OriginClear)

Another typical group in algae are the glycerol lipids, which are derived from fatty acids bound to a glycerol backbone. According to their structure – one, two or three aliphatic residues – they are named monoacylglycerols (MAG), diacylglycerols (DAG), or triacylglycerols (TAG). Polar lipids, typically phospho- and glycol-DAGs, play an important role as constituents of biological membranes forming a hydrophobic barrier to the environment and between cellular compartments. The most abundant are triacylglycerols that are the central component of the lipid energy catabolism in algae. Due to their non-polar nature, TAGs do not contribute to the osmotic potential of the cell and can accumulate in large quantities. Compared to starch, the specific energy content of TAGs is approximately twice as high and less rapidly mobilized. Based on the fatty acid composition, these storage lipids can be utilized for the production of biofuels – transesterification with methanol yields FAME (fatty acid methyl ester), which is used as Biodiesel – but also as food and feed additives or in pharmaceutical applications.

In spite of similar chemical nature, TAG's can be variable, differing by the level of saturation and the length of the aliphatic carbonyl chains. The differences vary between species, but can also be induced or increased in some species by different environmental conditions [32]. Only organisms reaching a high TAG or polyisoprenoids content are suited for biokerosene or diesel production.

Several strategies attempting to optimize lipid biosynthesis have been researched so far. In an unique attempt to explore biological diversity of aquatic algae for biofuel production, the US Department of Energy (DOE) screened 3,000 algal strains in the 1980s for their capacity to produce lipids [4]. Eventually the project was terminated and many important issues remained unresolved [33]. In some organisms, lipids can accumulate as a natural preferential form of energy storage (e.g. *Dunaliella salina* with ca. 54 % lipid/DW [34], *Botryococcus braunii* with more than 60 % lipid/DW [35], *Ochromonas danica* with 37 to 71 % lipids/DW [36, 37]).

The starch and lipid energy-storage pathways can be influenced in green algae by specific cultivation conditions (e.g. [38–41]). The accumulation of lipids occurs under distinct regimes.

1) Under favorable irradiance and temperature and with abundant supply of nutrients and CO_2, the algal cells grow and divide rapidly with most lipids targeted to membranes, particularly those constituting chloroplast as the main anabolic driver. No energy reserves are accumulated.
2) When starved, for example by nitrogen or sulfur depletion, the photosynthetic CO_2 fixation continues but is directed towards generating nitrogen- and sulfur-free reserves, such as starch or non-polar lipids [42].
3) Enhanced accumulation of triacylglycerols (TAGs) also occurs under conditions when the light is too strong and induces a partial photoinhibition of photosynthetic activity [43, 44]. Such high light intensities generate reactive oxygen species that act as signal molecules not only for the production of neutral lipids but often also for secondary carotenoids synthesis [45], a putative high value by-product of biofuel production. The secondary carotenoids are assumed to protect the chlorophylls by light shading and, thus limiting photoinhibition. They are localized in cytoplasmic lipid bodies [44] and sometimes in stroma of the chloroplast [46].

4) Phosphorus starvation can also be used for enhancing lipid production [47]. However, the onset of lipid production may be delayed due to luxury P-uptake of algae [48].

The effect of temperature on TAG accumulation in algae is less than that for light [32]. In this respect however, one ought to remember that a lower temperature leads to saturation of photosynthetic reactions in lower light levels and, thus, to potential early onset of light stress [49]. High salinity [50] or extreme pH [51] may also lead to enhanced lipid production. Until now, little has been achieved with attempts to genetically modify certain microalgae, as well as efforts to modify expression of key enzymes implicated in lipid synthesis, especially with a view to mass cultivation.

14.3 Growing Algae

The cultivation modes of currently commercially used microalgae [52] consist of variants of heterotrophic and photoautotrophic growth. Commonly used carbon sources in heterotrophic culture include various sugars and organic acids. Additional light supply to the algae culture is optional, since the energy within these feed molecules – which themselves have been produced photosynthetically elsewhere before – is being transferred into growth and biomass production in algae bioreactors. Examples of the heterotrophic large scale production of microalgae and their constituencies are companies such as Martek, Solazyme and Alltech Winchester (Table 14.1) [53].

CO_2 and HCO_3^- are the single photoautotrophic carbon sources, with compulsory light supply to fix carbon photosynthetically in photobioreactors (PBRs). Cultivation systems for photoautotrophic growth of algae with sunlight as the primary source of energy are wide-spread (Fig. 14.1). These systems are designed to capture solar irradiation, absorb CO_2, and nutrients, and to produce O_2 and algal biomass. Two major kinds of photobioreactors exist [54]: “open” and “closed” types. Open systems are those in which the algal suspension is partially in direct contact with the ambient environment, whereas closed systems essentially have no contact with the outside air and light does not impinge directly on the culture.

Typical open systems are raceway ponds [22], open stirred tank reactors [55] and thin layered sloping systems [17, 24, 38]. The culture light path reaches from a few millimeters in the thin layered sloping ponds to 0.5 m depth in others. Pond sizes can vary from a few m^2 up to more than 2,000 m^2. Various means are used to mix the cultures (e.g. by paddle wheel, pump or air lift). The design is simple and costs are relatively low, making open ponds the currently leading system for commercial biomass production. Yet the use and proper dissolving of additional CO_2 is complicated (loss to atmosphere). Evaporation and risk of contamination is high. Large scale examples are the commercially used ponds of Sapphire, USA, or the pigment-producing facilities of Algatech, Israel.

Closed systems can be tubular and of various configurations [56, 57], consist of vertical or horizontal plates, plastic foils, bags and many other variants with variable short light path (<50 mm). Materials may be glass or synthetic and the reactor enclosed in glass houses or plastic tunnels. Extreme designs exist, such as

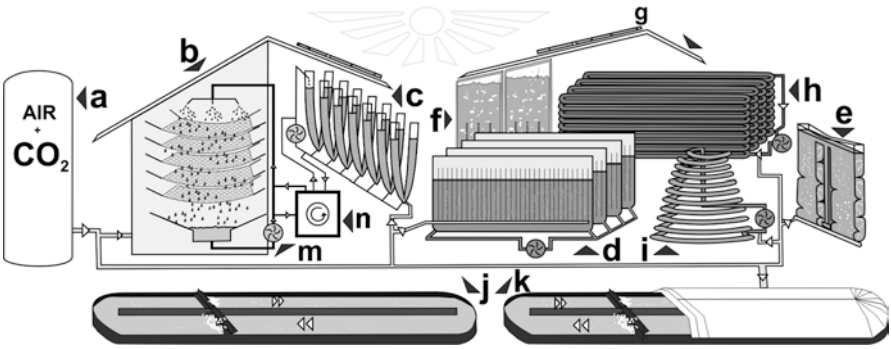

Fig. 14.1 Examples of algae photobioreactors currently used for microalgae production (The carbon source air/CO2 mix connected to all designs (a); Algae broth is sprayed through nets in CO2-enriched atmosphere for high culture density in closed greenhouse IGV (b); Synthetic bags for in- and outdoor use NOVAgreen (c), Phytolutions (d); Airlift-reactors e.g. Subitec (e) or façade flatpanel reactors BIQ (f); Synergistic effect may be achieved in combination with e.g. solar panels (g); Different types of tubular reactors, either made of glass (h) or synthetics GICON (i); Race-way-ponds, either open (j) or covered for increased protection and CO2 enrichment (k); Pumps (m); Exemplary harvest: centrifuge connected to algae circulation for continuous biomass harvesting (n))

horizontal layered nets on which the culture is sprayed and housed in an enclosure in which the atmosphere can be enriched with CO_2 [58]. Some systems have been incorporated in architectural designs (BiQ).

Advantages of closed over open systems are potentially higher light utilization efficiencies, reduced water losses and higher CO_2 sequestration, higher nutrient uptake (removal) and better resource management, lower compensation light/dark ratios or respiratory losses as well as reduced contamination with unwanted or harmful species, and therefore far higher volumetric biomass concentrations [54, 57, 59].

In contrast, photo-limited zones may exist (not enough or too much irradiation or overheating), dissolved oxygen levels can become too high, and biofouling and biofilm accumulation [60] through accompanying bacterial growth is common and requires frequent remediation. Most importantly, the investments and maintenance costs are very high and have to match the market value of the product.

Both “open” and “closed” systems can be used in multi-photobioreactor designs [54], exploiting local resources. This would also include the usage of waste products such as CO_2 (flue gas) nutrients (e.g. from sewage) and heat (e.g. industrial waste heat). The system designs may benefit from the same technology principles. For example, thin layer systems with a short light path across the algal suspension represent a significant advantage because they can support much higher cell densities, taking into account that the light absorption is governed by light path (layer thickness) and culture density. If an open thin layer system [61] is enclosed in a glass house or plastic foil enclosure with enriched CO_2 air, one can combine benefits of open and closed systems. At Forschungszentrum Jülich’s Algae Science Center,

IGV constructed a modification of this approach, in which the algal suspension is sprayed in droplets over a system of multiple stacked horizontal nets, enclosed in a CO_2-enriched atmosphere.

Multiple PBR concepts have been developed or are still under development at research facilities as well as start-ups world-wide, trying to bridge the gap between providing optimum environmental conditions to the culture and controlling the costs for maintenance and resources. Irrespective of the type of PBR utilized, aquatic cultures have a high water and fertilizer use, therefore resource management and water footprint reduction is important. An advantage of algal production systems is the possibility to recycle water and nutrients. A reduction of 84 % water and 55 % fertilizer has been reported [46] if harvest water is recycled instead of replacing it with fresh water as well as a 90 % water reduction if wastewater is used. Additionally, some algae species require saline conditions, such as *Nannochloropsis salina* [63] and *Dunaliella salina*. For such species, seawater or natural brackish water use is an option.

Furthermore, not all algae strains are suited for cultivation in closed PBR's, much less even in open systems. Understanding the specific growth requirements of a specific candidate species is the starting point of any large scale production system. Some species are better suited than others to out-perform co-existing organisms [64]. This always means finding a compromise between providing optimized conditions for the targeted organism (CO_2, light, temperature, culture density, nutrient composition, etc.) and still run the PBR at reasonable cost.

A new PBR can only be considered for a large scale production system if these compromises are working out. Additionally, large systems are needed as a prerequisite for biokerosene or diesel production to provide significant harvests for further processing. This implies that all laboratory and pilot scale systems aiming towards fuel products must be up-scalable. The transition to large-scale commercial production has its own challenges [54, 65] identified and related a number of variables that need to be optimized, including areal biomass density, turbulence, nutrient supply and control of predators, pathogens and alien microalgae invasion.

14.4 Harvesting Algae

Algal cultures as third generation biofuel source may be capable of yielding more biomass with less resources than other feedstock, but in contrast to crops used for first generation (derived from starch, sugar, or vegetable oil) or second generation biofuels (not derived from food crops – e.g. switchgrass or waste vegetable oil), they have a very low density before harvesting. Typical phototrophic algae cultures achieve a density of 0.5 to 2 g/L, corresponding to 0.05 to 0.2 % dw (m/v). Higher densities can be reached in specific photobioreactors, especially where the light path is thin (Table 14.1), or in cultures grown mixotrophically or heterotrophically. The higher the culture density is, the lower the costs of harvesting are. One or more of the steps described in the following can be employed for harvesting and subsequently for downstream processing.

Concentration of the cells can be achieved by filtration [66], flocculation e.g. by ferric chloride or similar [66, 67], light stress induced autoflocculation, electrocoagulation [68] or microwave synthesized magnetic iron particles [69], which cause the algae to coagulate; decanting or skimming then yields the flocs. An alternative is sedimentation/decantation [70] or flotation, which is achieved by various means of aeration [70].

Centrifugation can be used to separate the biomass from the growth medium or to further dewater a pre-concentrated algal biomass. Centrifugation is rather energy consuming, and energy use of more than 1 kWh/m³ have been reported [66, 68]. More efficient centrifuge types are being developed, e.g. hydrocyclones [66] or spiral plate centrifuges [68]. A pre-concentration step (see previous paragraph) can further improve the energy balance of harvesting [68].

Some processes in the downstream require drying, which can be achieved through simple evaporation, solar drying, freeze drying or spray drying, the latter two being energy intensive and expensive, but yield high quality products [66]. Independent from the analyzed pathway, harvesting remains the cause of the major expenses.

14.5 Extraction and Converting Algal Lipids into Biokerosene or Biodiesel

Usually, decomposition of the microalgae cells is required to access the oil [66, 71]. Several strategies have been followed to access or separate the oil from the other components (i.e. physical pretreatment (ultrasound, milling or mechanical sheer forces), extraction with supercritical CO_2, extraction with organic solvents or thermal conversion (hydrothermal liquefaction HTL) [72].

Pretreatment enhances the efficiency of extraction by disrupting the cellular structure, releasing lipids into the solvent mixture, and enhancing overall yield. Dewatered algae can be dried and milled into a fine powder. Alternatively, microwaving, chemical lysis, or high-pressure homogenization is applied to increase the mass transfer of lipids during extraction.

Oil of the microalgae can be extracted using n-hexane, chloroform, benzene, diethyl-ether or ethanol. N-hexane is the most common solvent. An advantage of using these solvents is that they are inexpensive, very efficient and typically used for oil extraction [71]. Carotenoids and highly unsaturated lipids are extracted commercially with supercritical CO_2. Both methods leave behind a lipid free biomass for further processing – e.g. of the residual carbohydrates.

Alternatively, thermal conversion processes such as hydrothermal liquefaction (HTL) convert the whole biomass (10 to 15 % dry weight), including carbohydrates and proteins, through high pressure and temperature into four streams; i.e.

1) non-aqueous biocrude (composed primarily of, phenolic compounds, and long-chain alkanes) (20 to 60 wt%),
2) an aqueous phase containing organic acids and most of the soluble nitrogen- and phosphorus-salts in the biomass (30 to 50 wt%),

3) a gas-phase containing CO_2, CH_4, and volatile organic compounds (1 to 8 wt%), and
4) a solid phase consisting primarily of biochar (~3 wt%) [73].

A maximum oil yield of 25 to 44.8 wt% at 300 to 360 °C and 10 MPa was reported [74–76]. Recent studies on the economic feasibility of cultivating algae show that a multi-product approach will significantly increase the economic potential and the competitiveness of the process [8].

After extraction, the crude oil consisting of triacylglycerol (TAG) and also free fatty acids (FFA), terpenes and proteins must be refined to yield biofuel. For biodiesel, transesterification is the preferred solution, turning TAGs and FFAs into FAME, the final product "biodiesel". FAME however does not meet the criteria for high performance fuels like kerosene [77]. Converting the biodiesel to kerosene or diesel-like hydrocarbons includes a cracking step to modify the length of the hydrocarbons and a hydrogenation step to remove oxygen and unsaturated C=C bonds. Both steps can either be separated (fluid catalytic cracking, thermal cracking) or combined (hydrotreating/hydrocracking), eventually yielding a non-oxygenated fuel, which is chemically identical to fossil fuels like diesel or kerosene.

14.6 Final Considerations

Initiatives and Global Activities. Currently, big players (e.g. Solazyme, Sapphire Energy, Algenol) aim to produce on industrial scale, delivering or intending to deliver algae-derived crude oil and tailored fuels to the market. These activities are mainly supported by national funding programs. Additionally, the number and diversity of other companies and groups entering the market grows constantly. For example, Honeywell UOP uses well-established refinery technology of deoxygenation, isomerization and cracking to produce renewable jet fuels from various biomasses, including algae. Other companies connected to fuel production are Universal Oil Products, AlgaFuel, Algae.Tec, Bio Fuels, Blue Marble Production, Diversified Technologies, Genifuels, GreenFuel Technologies, NOVAgreen, Origin Oils, Phytolutions, Proviron, Seambiotic, Synthetic Genomics, LS9 and numerous others. They are often involved in collaborative actions with research and development.

The need for alternate sustainable transport energy production is a major concern and various role players and NGO's are getting involved, such as The National Algae Association (NAA), a US non-profit organization of algae researchers, companies and the investment community or the Algae Biomass Organization (ABO), who seek alternative feedstock for biofuel markets covering the whole value added chain of algae. In Europe, the European Algae Biomass Association (EABA) promotes the use of algae (not just fuel) for research and industrial applications. The US Department of Energy is supporting a consortium of universities and companies who focusses on algae feedstock supply, feedstock logistics, as well as on conversion and production pathways concerning liquid transportation fuel production. The US military invests in large-scale oil and jet fuel production from algae, including refining the fuels. The "Producing biofuels from marine algae" research program,

launched by the National Research Council Canada (NRC Canada) is investigating technical and biological aspects, and the Algal Carbon Conversion (ACC) Flagship Program of the NRC, in association with Canadian Natural Resources and Pond Biofuels, aims to develop a pilot-scale algal biorefinery. There is also an Australia/New Zealand initiative for producing sustainable aviation fuel called "Flight path to green aviation".

The importance of sustainable transportation fuel is also recognized in Europe. Initiatives of the European Union (EU) are aligned with the EU's ambitious renewable energy targets, and under the Algaecluster there are initiatives such as BIOFAT, All-Gas and InteSusAl.

On a national level, the UK Roadmap for Algal Technologies was commissioned by the UK Natural Environmental Research Council Algal Bioenergy Special Interest Group. Besides environmental implications, it pays attention to the economic impact of algae-related products, processes and services for the UK. The Netherlands launched an algae research program called "Towards Biosolar Cells". An important research and experimental facility in the Netherlands is AlgaePark is Wageningen. In Germany there is a similar research initiative at the Algae Science Centre (AlgaeSC), Forschungszentrum Jülich. The need for alternative biofuels and CO_2 footprint reduction has been recognized and is tackled globally, not least focusing on algae.

Perspectives. The potential of microalgae as a biomass source of the future can be attributed to the fact that they are more productive than higher plants, require fewer nutrients compared to known energy crops, may be grown on fresh water or salt water, do not require agricultural land and can directly utilize CO_2. Microalgae have shown to be a source for a wide spectrum of fuel products in pilot studies (e.g. biodiesel, biomethane, bioethanol, green diesel, gasoline, kerosene, hydrogen [78]). However, with present technology, fuel production from microalgae is not yet economically viable and according to [8] it will remain like this for another decade (see also [79]). In consequence, research is important and rigid data are needed to refute false claims [80]. Economic viability will depend on utilizing all fractions of the biomass, process optimization and sensible application of synergies and waste resources. Finally, of course, the price of fossil crude oil is an important benchmark to meet – or at least approach.

Taking the production balances into account, most PBR systems are not efficient yet. The net energy ratios (NER) of PBR systems tested in Spain, are all greater than 1 according to [21, 81]. The same is true for other PBR's [24, 21]. Best performing PBR was a flat-plate system, which outperforms tubular PBRs as it benefits from a large illumination surface area and low oxygen build-up [81]. A comparison between a raceway pond biomass production and an idealized tubular PBR resulted in a price of ~1.6 to 1.8 €/kg versus ~9 to 10 €/kg, respectively [81]. Most of the production costs in raceway systems are associated with operation (labour, utilities and raw materials). In contrast, the cost of production in closed PBRs is dominated by the capital cost. This would most certainly converge with successful upscaling. To date, estimates are mostly based on extrapolations from laboratory-scale experiments, leading to large inaccuracies. Productivity is often overestimated and factors

like biological interaction and competition or geographical location are vastly underrated. A maximum lipid yield of 24 to 27 m³/(ha a) has been estimated using model simulations for 4,388 locations around the world [82].

Options: Bioprospecting, Strain Development and Breeding. Table 14.1 shows that the number of algal species currently used in large scale cultivation facilities is small, compared to the approximate 33,000 names in the AlgaeBase and estimated 72,500 species [83] (for an extensive review of procedures and strategies in bioprospecting for new lipid-producing algae see [84]).

Properties that are important for fuel production are a high cellular content of non-polar lipids, high growth rates, and resilience to shear stress. Finding potent and robust organisms in nature ought to be followed by a phenotypic and genetic characterization and a lipid-targeted domestication of the wild-type organisms. In comparison, domestication of wild plants into modern crops took approximately 13,000 years of selection aiming at a particular desirable characteristics (see e.g. [85]).

Since screening is crucial, the manipulation of growth conditions is important, such as exposing the candidate species to extreme temperature, light, unfavorable changes in pH or nutrients and propagating the survivor cells. Mutagenic chemicals can be applied and model organisms like *Chlamydomonas reinhardtii* may already be genetically manipulated towards a desired characteristic [86, 87] (for a review of the perspectives of targeted metabolic engineering towards new genetically modified algae see [88] and [89]).

Conventionally, cells with an elevated lipid content are identified by fluorescent dyes like Nile red [90] or BODIPY 505/515 [91]. An advantage of these staining methods (such as Fluorescence Activated Cell Sorting and Flow Cytometry [92, 93]) can be used to pick the cells with high lipid contents. Another method for isolating lipid rich algae is Raman micro-spectroscopy, that not only sense lipids in individual algal cells but also reveal their typical iodine number [94]. Also ^{1}H and ^{13}C NMR-spectroscopy (Nuclear Magnetic Resonance) (it was recently shown that lipids can be detected on the level of individual cells [95]) help to perform a successful selection of strains with desired traits.

Options: Added Value by Waste Utilization and Bioremediation. A lucrative option of improving the economics of algal biomass and biofuels production is the potential to use waste streams [96]. Waste CO_2 is produced by many processes (e.g. burning fossil fuels in power plants, cement production, waste water treatment [97, 98]). Utilizing waste CO_2 to produce algal biomass has many benefits (i.e. contributing to bioremediation and limiting one of the most costly items in producing biomass from microalgae [99]). In general the conversion rate of CO_2 into algal biomass is about 2 kg CO_2 per kg biomass, whereas the utilization efficiency is better in closed PBR than in open reactors. Several techniques are applied to open systems that improve the CO_2 absorption, such as sparging in wells or floating a transparent plastic sheet on the surface of the spargers [100]. CO_2 can also be utilized from flue gas of power generation plants. By combining a microalgae production plant that utilizes waste CO_2 and produces biomass, which is then further

utilized, a neutral carbon cycle is established. Vast areas of land would be required to treat even the CO_2 release from only a small sized power plant – it could however be complimented by other CO_2 absorption processes [101].

The basic benefits of using algae in wastewater treatment were recognized already more than 50 years ago [102], where microalgae could be used to treat wastewater and recover the nutrients and CO_2, to produce biomass in an aerobic system. These earlier High Rate Algal Treatment Plants (HRAP) systems had a number of issues, one of which was odor. The HARP systems were replaced with Advanced Integrated Wastewater Pond Systems (AIWPS) where, amongst others, microalgae are used at two stages of the process and excessive organic carbon is converted to methane [103, 104].

The next step was to combine wastewater treatment with biofuel production [105, 106]. Relevance of the synergistic technologies for perspectives of algal biofuels can be demonstrated in detail on the example of sequestering waste water phosphorus to produce algal lipids and P-rich post-extraction residue. P-fertilizers represent a foundation of modern agriculture and are a pre-condition for food safety of human mankind [107], but mineral P is a finite source and becomes more and more scarce [108, 109]. The post-extracted algal residue [110] contains P-rich matter that can be utilized as a slow release fertilizer [111–113], representing a promising co-product of algal biofuel production.

Present Situation and Outlook. The statement by Richmond [55] that "Microalgae cultures, however, is yet far from supplying any basic human needs, and the major reason for this stems from failure to develop production systems which utilize solar energy efficiently", is as relevant today as 20 years ago. However, some progress has been made, especially in terms of understanding the controlling factors in various PBR and the physiological properties of microalgae grown in outdoor reactors [114, 115].

It has been stated many times that the area productivity of algal cultivation systems is significantly higher than with higher plant crops [6] (Table 14.1), and it has been estimated that with present technology the area productivity of algae can be about 20,000 L(lipids)/(ha a), whereas it is between 1,500 and 6,000 L(lipids)/(ha a) for higher plant crops with algae not being dependent on arable land, minimizing competition with food crops [8].

But at present, liquid fuels production from microalgae is far too expensive compared to petroleum fuels. According to [116] fuel from algae would only be economically viable in a scenario where crude oil prices exceed 100 US-$/bbl [117]. In theory, high oil species of microalgae cultured in growth optimized conditions of PBRs have the potential to yield 10 to 27 t/(ha a) of microalgae oil [82]. The downstream processing pathway does not change whether biomass is produced in fermenters, closed systems or open ponds. Accordingly, the cost of biomass production is of high importance for the whole balance. It has been estimated that for a production of 10,000 t/a, the cost of the biomass for PBRs and raceways would roughly be 0.47 and 0.60 US-$/$kg_{DM}$ [116], respectively. This means that at 30 % lipid content for PBRs and raceways a liter of oil would cost roughly 1.40 and 1.81 US-$,

respectively. Production cost in closed PBR can be reduced by more than half if the production is scaled from 1 to 100 ha [8,79].

The data base for such assumptions is still thin, especially considering that the production parameters change heavily between different locations. Establishing a wider data base for future reference is crucial. It will take time to fully explore the huge potential of products from microalgae. To date, it can be stated that growing microalgae implies multi-facetted and species-tailored approaches. Microalgae production towards biofuel is not yet, and will only become, economically feasible if all currently possible synergies are exploited and parallel produced high value compounds accompany biofuel production. If cost and energy efficient cultivation can be achieved, microalgae have the potential to outperform any known crop. Accordingly algae must be further investigated with preference to enhance production, close the knowledge gaps and improve scalability and automation.

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