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MICROALGAE BIOFUEL POTENTIALS (REVIEW)

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With the decrease of fossil based fuels and the environmental impact of them over the planet, it seems necessary to seek the sustainable sources of clean energy. Biofuels, is becoming a worldwide leader in the development of renewable energy resources. It is worthwhile to say that algal biofuel production is thought to help stabilize the concentration of carbon dioxide in the atmosphere and decrease global warming impacts. Also, among algal fuels' attractive characteristics, algal biodiesel is non toxic, with no sulfur, highly biodegradable and relatively harmless to the environment if spilled. Algae are capable of producing in excess of 30 times more oil per acre than corn and soybean crops. Currently, algal biofuel production has not been commercialized due to high costs associated with production, harvesting and oil extraction but the technology is progressing. Extensive research was conducted to determine the utilization of microalgae as an energy source and make algae oil production commercially viable.

The world's demand for energy is steadily increasing. Global demand for petroleum is predicted to increase 40% by 2025 [1]. So, the use of an alternative fuel becomes necessary. Biofuels are a wide range of fuels which are in some way derived from biomass. The term covers solid biomass, liquid fuels and various biogases. It is expected to expand biofuels to 36 billion gallons by 2022. Third generation biofuels ("advanced biofuel" or biodiesel from microalgae) are a promising alternatives to other biofuels but there are still vagueness to be investigated. Their energy output per land unit is at least 30 times higher than for 2nd generation biofuels [2]. Biodiesel defined as a mixture of monoalkyl esters derived from fatty acids of oil crop or animal fats is biodegradable, and nontoxic. Biodiesel in conventional diesel engines reduces emissions of unburned hydrocarbons, carbon monoxide, sulfates, polycyclic aromatic hydrocarbons and nitrated polycyclic aromatic hydrocarbons. Microalgae appear to be the only source of renewable biodiesel that is capable of meeting the global demand for transport fuels [3]. Each of the three biochemical fractions of microalgae (lipids, carbohydrates, and proteins) can be converted into fuels. The lipids of some species are hydrocarbons, similar to those found in petroleum, while those of other species resemble seed oils, can be converted to a synthetic diesel fuel (ester-fuel) by transesterification. Carbohydrates are commonly converted to ethanol by fermentation. Alternatively, all three fractions can be converted to methane gas by anaerobic digestion [4]. Oil content of some microalgae may exceed 80% of the dry weight of algae biomass. Even microalgae of low oil content can produce ten times the amount of the most productive terrestrial biodiesel feedstocks. Algae are said to yield about 1.200–10.000 gallons of oil/acre, compared to 48 and 18 gallons/acre for soy and corn, respectively [5, 6]. The major threshold for producing microalgal biodiesel is the high cost of raw material (US$2.4/l microalgal oil), which is 3–4 times higher than plant oil. Algae can be grown in ponds, closed photobioreactors or in plastic tanks called bioreactors. Over 90% of the world's commercial microalgae production uses shallow, open, paddle wheel mixed, raceway type ponds but closed photobioreactors represent only about 1%. The approximate number of companies directly involved in producing fuels from the algae is increasing with a high rate as 50 in 2008 to 75 in early 2009, 100 in mid 2009 and 150 at the end of 2009. Only about 10000 metric tons of microalgal biomass (dry matter basis) is produced annually in commercial operations, with a typical selling price of from 5000 to over 100.000$ US per dry t of biomass. When formulated into finished consumer products, this biomass generates a turnover of several billion dollars per year. This article focuses on microalgae as a potential source of biofuel, cultivation, biofuel opportunities and attempts towards commercial algal fuel production [5].

WHY BIOFUEL? WHY ALGAL BIOFUEL?

The remaining global reserves of crude oil are continuously declining. Based on the available data, the world will run out of crude oil in 24 to 57 years from today [7]. It was estimated that by 2050 biomass could provide nearly 38% of the world's direct fuel use and 17% of the world's electricity [8]. A major criticism against large-scale biofuel production is that it will occupy vast farm land and native habitats, drive up food prices, and result in little reduction in CO_2 emissions. At the moment, only biodiesel and bioethanol are produced on an industrial scale which are derived from food crops such as sugarcane, sugar beet, maize (corn), sorghum and wheat which is known as first generation biofuel. The basic feedstocks for the production of first-generation biofuels are often grains, which yield starch that is fermented into bioethanol, or seeds, which are pressed to yield vegetable oil that can be used in biodiesel. Second generation biofuels use biomass to liquid technology, including cellulosic biofuels (grassoline) from nonfood crops, including waste biomass, the stalks of wheat, corn, or wood. These biofuels are inherently more efficient than first generation technologies because they use more of the plant to produce fuel [3]. Third-generation biofuels "advanced biofuels" are derived from algae [3]. For 2010 it is expected to have 100000 gallons of algal biofuel production which will increase to 6 billion gallons in 2025 [9]. It is said that in 2020, 30% of algae production goes to oil fraction [10]. Algal biofuels have a tremendous variety. Algal biomass may be used directly as a solid biofuel to generate heat, steam and electricity or converted to gaseous biofuels, such as biogas and biohydrogen. Algal biomass rich in starch can be easily fermented to liquid biofuels such as bioethanol and biobutanol. Algal oils can be converted to diesel, gasoline and jet fuel using existing technology [5]. Currently biodiesel is used for blending (2–10%) with crude oil without the need for any modifications in existing engines since that makes no difference in vapour pressure, viscosity, density, and octane/cetane number. Algae can produce 267 l of ethanol (assuming a 40% starch content) and 190 l of biodiesel/ dry t [11]. Microalgae containing 30% oil by weight of dry biomass could yield almost 587000 l/ha or 5.000–15000 gallons/year [10]. However, up to now, the commercial production of biofuels from microalgae has not been realised on an industrial scale in a cost-efficient manner. Several problems have arisen during the large scale production, including high investment costs for production facilities and energy demand for harvesting biomass of low concentration [12]. Microalgal biofuels are 4–10 times as expensive to produce as petroleum-derived fuels or other biodiesels [5].

STRAIN SELECTION

Looking for the microalgal strains with the combination of high oil content and a rapid growth rate is the start of biodiesel production. A limited number, about 4000 species have been identified, which can be divided into several groups including cyanobacteria (Cyanophyceae), green algae (Chlorophyceae), diatoms (Bacillariophyceae), yellow-green algae (Xanthophyceae), golden algae (Chrysophyceae), red algae (Rhodophyceae), brown algae (Phaeophyceae), dinoflagellates (Dinophyceae) and 'pico-plankton'(Prasinophyceae and Eustigmatophyceae) [13]. The three most prevalent groups of algae targeted for biodiesel production include the diatoms that make up a majority of phytoplankton in salt and brackish waters, green algae common in many freshwater systems, blue-green algae (Cyanophyceae), which are actually bacteria that contain chloroplasts and are important to nitrogen fixation in aquatic systems, and finally the golden algae species able to store carbon as oil and complex carbohydrates [14–16]. They have oil levels between 20 and 75% by weight of dry biomass (Table 1). In general, lower oil strains grow faster than high oil strains [17]. Microalgae containing 30% oil grow 30 times faster than those containing 80% oil [18]. Another challenge is that microalgae usually accumulate oil under stress conditions with slow growth rate. The composition of microalgal fatty acids has a significant effect on the fuel properties of biodiesel produced. The proper percentage of saturated and unsaturated fatty acid is very important to microalgae as a biodiesel feedstock [10].

For strain selection, some factors are: lipid content, more the distribution of free fatty acids and triglycerides not only the total lipids; resistance to environmental conditions changes, competition from other microalgae species and/or bacterial; nutrients availability; ease of biomass separation and processing; possibility of obtaining other valuable chemicals. Even when the species are not quite deasirable for the purpose in commercial use, the utilization of genetic engineering may be a solution [21]. Type of metabolism is also important for strain selection. Microalgae may assume many types of metabolisms (autotrophic, heterotrophic, mixotrophic, photoheterotrophic) and are capable of a metabolic shift as a response to changes in the environmental conditions [22]. Generally, heterotrophic cultivation has been found to increase the total lipid content in algae compared to phototrophically grown cells. Mixotrophic, perform photosynthesis as the main energy source, though both organic compounds and CO_2 are essential. Amphitrophy, a subtype of mixotrophy, means that organisms are able to live either autotrophically or heterotrophically, depending on the concentration of organic compounds and light intensity available. For species that can utilize both light energy and chemical substrates, this mode of cultivation offers a superior alternative to phototrophic and heterotrophic growth. Photoheterotrophic, also known as photoorganitrophy, photoassimilation, photometabolism, describes the metabolism in which light is required to use organic com-

Table 1. Lipid content of some microalgae [18, 19]

Microalgae species	Lipid content, % dry waight biomass	Microalgae species	Lipid content, % dry waight biomass
Ankistrodesmus species	28–40	*Euglena gracilis*	14–20
Anabaena cylindrica	4–7	*Ellipsoidion* sp.	27
Botryococcus braunii	25–86	*Haemotococcus pluvialis*	25
Chaetoceros muelleri	33	*Hantzschia* species	66
Chlamydomonas species	23	*Isochrysis galbana*	21.2
Cllorella emersonii	25–63	*Monallantus salina*	20–22
Chlorella minutissima	57	*Nannochloropsis* sp.	20–56
Chlorella protothecoides	14–57	*Neochloris oleoabundans*	35–65
Chlorella sorokiniana	22	*Nitschia closterium*	27.8
Chlorella vulgaris	14–56	*Nitschia frustulum*	25.9
Crypthecodinium cohnii	20–51	*Pavlova lutheri*	35
Cyclotella species	42	*Phaeodactylum tricornutum*	20–30
Dunaliella primolecta	23	*Prostanthera incisa*	62
Dunaliella salina	28.1	*Prymnesium parvum*	22–39
Dunaliella tertiolecta	36–42	*Pyrrosia laevis*	69.1
Skeletonema costatum	13–51	*Spirulina plantensis*	16.6
Scenedesmus dimorphus	16–40	*Stichococcus* species	33
Scenedesmus quadricauda	19.9	*Tetraselmis suecia*	15–23
Schizochytrium sp.	50–77	*Thalassiosira pseudonana*	20
Selenastrum species	21.7	*Zitzschia* sp.	45–47

pounds as carbon source. The photoheterotrophic and mixotrophic metabolisms are not well distinguished, in particular they can be defined according to a difference of the energy source required to perform growth and specific metabolite production [5].

MICROALGAE CULTIVATION

When inoculating a batch of microalgae, subculture is usually used to ensure continuous growth and division of cells. Water, air and carbon dioxide stream should be filtered to reduce contamination risk. Cultivation can be conducted in batch, semi-batch, and continuous systems. Batch culture consists of a single inoculation of cells in container of media over several days of growth period until the cell density reaches a maximum/desirable level ready to be transferred to larger culture volumes to continue growth before reaching the stationary phase. The semi-batch system allows a portion of the culture to be harvested and replenished with fresh medium. In a continuous system, two types of culture can be used: turbidostat and chemostat culture. In a turbidostat culture, when the density reaches a preset level, fresh medium is added to the culture as the cells continue to divide and grow. In the chemostat culture, a slow but steady flow of fresh medium is continually introduced into the culture while excess culture overflows and collected. Two types of reactors have been developed to cultivate algae: open system (such as raceway ponds) and closed system (such as photobioreactors).

Open pond systems. Such systems can be excavated and used unlined or lined with impermeable materials, or they can be built up with walls. Sometimes unlined ponds are used to reduce costs, but they suffer from silt suspension, percolation, heavy contamination, and their use is limited to a few algal species and to particular soil and environmental conditions [23]. Raceway ponds are open, outdoor ponds that are made of circulating loop channels and are typically shallow (less than 0.3 m deep) and unlined. Open pond has moderate surface-to-volume ratio of 3–10/m [24]. Paddle wheels are used to circulate the suspended algae throughout the raceway channels. Cooling is mostly done by evaporation, and the pond is illuminated solely by sunlight. The raceway pond can be run continuously with growth medium and carbon dioxide feed continuously added to the pond while algal broth is harvested at the end of the circulation loop. Production in the pond usually takes 6–8 weeks to mature and typically yields only 0.1–0.2 g/l algae [25]. Open ponds are dependent on weather because temperature and light intensity vary throughout the day and year. Low temperatures (<17°C) reduce algal growth rate while high temperatures (>27°C) kill algal cells. If cultivation is a success, high biomass yields are mostly

seasonal. Tank size also influences algal growth. Smaller outdoor ponds produce higher algal yield than a larger pond. The open pond system can be converted to an indoor system by covering the pond with a layer of plastic or glass [23]. Raceway pond is the most commonly used design as an open culture system [5]. Open ponds are only suitable for a small number of algal species that can tolerate extreme environmental conditions. The size of commercial ponds varies from 0.1 to 0.5 ha. Raceway ponds are widely used for the commercial cultivation of *Spirulina*, *Haematococcus* and *Dunaliella* [26]. The productivity of raceways is almost 10 times higher than unmixed algae ponds. Open systems can be easily scaled up to several acres for individual ponds. Currently, 98% of commercial algae are produced in open systems [27]. Circular ponds are the other type of open pounds used mainly in Asia for the production of *Chlorella*. These ponds are mixed by a centrally located rotating arm (similar to those used in wastewater treatment). Thin layer, inclined ponds consist of slightly inclined shallow trays, over which a very thin layer of algae flows to the bottom where the culture is collected and returned to the top. Mild mixing or wave producer such as paddlewheel, waterjet or air pump systems can be used to make a water velocity typically at 30 cm/s (much higher velocities require excessive amounts of mixing energy) [28]. Unmixed open systems are not true algae production ponds because production is not maximized and the biomass produced is rarely harvested. Even when the biomass from unmixed ponds is harvested, their chemical byproducts interfere with utilization for biofuels [29].

Photobioreactors (PBRs). PBRs were developed to overcome the problems associated with open pond systems. They can be located indoors and provided with artificial light or natural light via light collection and distribution systems or outdoors to use sunlight directly [30]. PBRs can be classified on the basis of both design and mode of operation. Reactors can be tilted at different angles and can use diffuse and reflected light, which plays an important role in productivity. Materials such as plastic or glass sheets, collapsible or rigid tubes, must lack toxicity, have high transparency, high mechanical strength, high durability, chemical stability and low cost [31]. Closed systems like photobioreactors have higher efficiency and biomass concentration (2–5 g/l), shorter harvest time, reduce contamination risk, and allow greater selection of algal species used for cultivation and higher surface-to-volume ratio (25–125/m) than open ponds [32]. Light can be radiated inside the bioreactor with optical fibers or submerged lamps, or provided externally by fluorescent lights or the sun. The photobioreactor has a photolimited central dark zone and a better lit peripheral zone close to the surface [5]. Carbon dioxide enriched air is sparged into the reactor creating a turbulent flow which circulates cells between the light and dark zones and assists the mass transfer of carbon dioxide and oxygen gases. The frequency of light and dark zone cycling is depended on the intensity of turbulence, cell concentration, optical properties of culture, diameter of tube, and the external irradiance level [5]. Regulation of carbon dioxide and dissolved oxygen levels in the photobioreactor is another key element to algal growth. Challenges with closed system photobioreactors include overheating, build up of photolimited zones in the inner zone, photoinhibition in the peripheral zones, cell structure damage due to hydrodynamic stresses, and growth on the reactor wall [5, 23, 33] and cost. The scale-up of bioreactors increases the percentage of dark zone and reduces algal growth. The highest cost for closed system is the energy cost associated with the mixing mechanism [34]. A small scale bioreactor can be easily incorporated into a pilot plant as an indoor or outdoor system. The most popular photobioreactors are as follows: tubular systems [35–38]; helical PBRs [30]; plastic bag systems [28]; well systems; pyramid photobioreactors; airlift photobioreactors [39]; annular photobioreactors [40]; column photobioreactors [39–41]; bubble column photobioreactors [41]; vertical column photobioreactors [36, 42]; flat Plate PBRs [38, 43, 44]; stirred tank photobioreactors [41]; rectangular tanks [45]; immobilized bioreactors [46–50]; hybrid systems [51].

In conclusion, PBR and open ponds should not be viewed as competing technologies, but the real competing technology will be genetic engineering [31]. PBR can be operated in batch or continuous mode. There are several advantages of using continuous bioreactors instead of the batch mode (Table 2) [52]: continuous bioreactors provide a higher degree of control than do batch, growth rates can be regulated and maintained for extended time periods and biomass concentration can be controlled by varying the dilution rate, because of the steady-state of continuous bioreactors, results are more reliable and easily reproducible and the desired product quality may be more easily obtained.

Fermenters. They are similar to bioreactors in that they are closed or semi closed systems used for the production of biomass. However, fermentors utilize an organic source of carbon (sugar) as the source of energy and carbon instead of light and photosynthesis. Fermentors can usually achieve much higher biomass than a photobioreactor, but the cost per unit weight is usually much higher due to cost of supplying the fixed carbon source [28].

Closed photobioreactors are recommended for scaling up of microalgae because they can be erected over any open space, can operate at high biomass concentration, can keep out atmosphere contaminants and can save water, energy, and chemicals compared to some other open culture systems. The biomass productivity of photobioreactors can average 13 times more than that of a traditional raceway pond. A combination of open ponds and closed photobioreactors is probably the most logical choice for cost-effective cul-

Table 2. Open pond cultivation of microalgae vs. photobioreactors [33, 53, 54]

Factor	Open pond	Photobioreactor
Required space	High	For PBR itself low
Water loss	Very high, may also cause salt precipitation	Low
CO_2-loss	High, depending on pond depth	Low
Oxygen concentration	Usually low enough because of continuous spontaneous outgassing	Build-up in closed system requires gas exchange devices (O_2 must be removed to prevent inhibition of photosynthesis and photo oxidative damage)
Temperature	Highly variable, some control possible by pond depth	Cooling often required (by spraying water on PBR or immersing tubes in cooling baths)
Shear	Usually low (gentle mixing)	Usually high (fast and turbulent flows required for good mixing, pumping through gas exchange devices)
Cleaning	No issue	Required (wall-growth and dirt reduce light intensity), but causes abrasion, limiting PBR lifetime
Contamination risk	High (limiting the number of species that can be grown)	Medium to low
Biomass quality	Variable	Reproducible
Biomass concentration	Low, between 0.1 and 0.5 g/l	High, generally between 0.5 and 8.0 g/l
Production flexibility	Only few species possible, difficult to switch	High, switching possible
Process control and reproducibility	Limited (flow speed, mixing, temperature only by pond depth)	Possible within certain tolerances
Weather dependence	High (light intensity, temperature, rainfall)	Medium (light intensity, cooling required)
Start-up	6–8 weeks	2–4 weeks
Capital costs	High ~ US$100000 per ha	Very high ~ US$250000 to 1000000 per ha (PBR plus supporting systems)
Operarting costs	Low (paddle wheel, CO_2 addition)	Higher (CO_2 addition, oxygen removal, cooling, cleaning, maintenance)
Harvesting costs	High, species dependent	Lower due to high biomass concentration and better control over species and conditions

tivation of high yielding strains for biodiesel [53]. In the first stage, the microalgal strain with high oil content is grown in photobioreactors to produce biomass. In the second stage, the microalgae enter an open raceway with nutrient limitations and other stressors to promote biosynthesis of oil.

Once an algal culture reaches maturity, the biomass is harvested from the culture medium. Biomass harvesting may be one of the more contaminating processes in the production of algae-based biofuels. There are three systemic components of the harvesting process: biomass recovery, dewatering, and drying. The costs of harvesting can be a significant proportion of the total algal production costs, ranging from 20 to 30% [55]. In order to produce energy from algae as economically as possible, the cheapest way of concentrating the algal biomass low enough for oil pressing is essential. The technically simplest option is the use of settling ponds which is filled with a fully grown algae culture and drained at the end of that day, leaving a concentrated biomass volume at the bottom, which is stored for further processing [56]. There are other efficient techniques for recovering algal biomass, the implementation of which may vary depending on existing pond conditions or PBR design. They are as follows: Flocculation (induced in various ways, such as chemical flocculation, bioflocculation, electroflocculation); dissolved air floatation; centrifugation, filtration, decantation and vacuuming, dewatering, and drying [3, 19, 55, 57–59].

BIOFUEL POTENTIALS

Algae for biofuels have been studied for many years for production of hydrogen, methane, triglycerides for biodiesel, hydrocarbons and ethanol. Methane was the

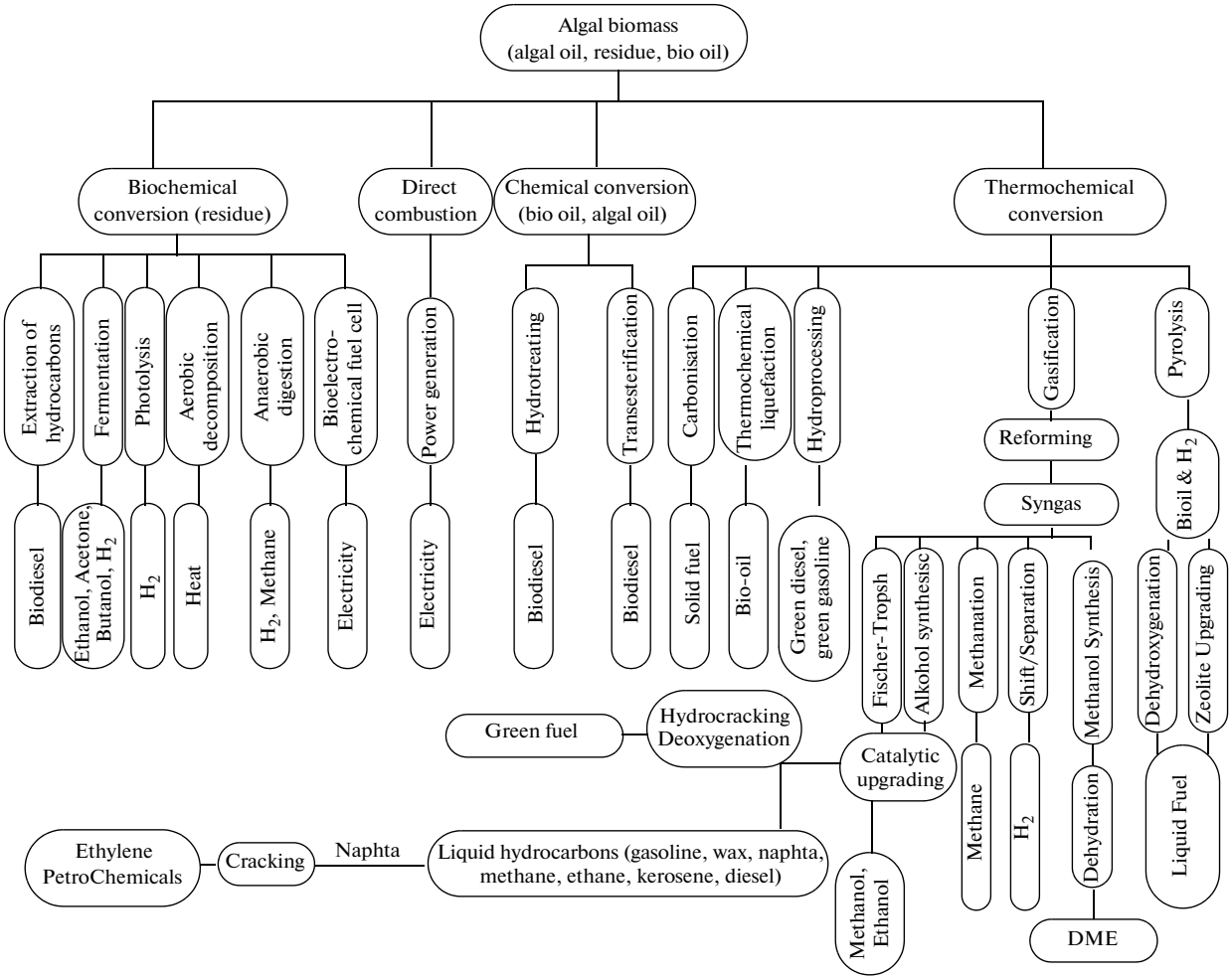

Fig. 1. Algae biomass transformation to energy [3].

focus of the early work in microalgae biofuels production. Since the 1980s, after the first oil shocks and higher value of liquid transportation fuels focused are on algae oil, specifically biodiesel production. Conversion processes of algal biomass (or components of biomass) into several possible biofuels and coproducts are of varying efficiency depending on reaction temperature, pressure, heating rate, and catalyst type, as well as algal species and quality of biomass. If oil extraction is done on algae the product would be algal oil. If thermochemical pretreatment is done on algal biomass bio-oil will be derived that is different from algal oil. They will play as intermediate products in determining which processs may be used to get different kind of fuels [3, 60] (Fig. 1).

Hydrocarbon. Hydrocarbons are fuels such as gasoline, diesel, and jet fuel that do not contain oxygen but carbon and hydrogen. Bioderived hydrocarbon fuels are products of thermochemically converted algal oil or bio-oil and are sometimes referred to green or renewable gasoline, diesel, naptha and jet fuels. Methane (CH_4), ethane (C_2H_6), and propane (C_3H_8) are the most famous ones [3]. *Botryococcus braunii* is well known for its ability to produce hydrocarbons which have been loosely described as equivalent to the "gas-oil fraction of crude oil" [61]. Like petroleum, these hydrocarbons can be turned into gasoline, kerosene and diesel. While other algal species usually contain less than 1 percent hydrocarbons, in *B. braunii* they typically occupy 20–60% of its dry matter, with a maximum of >80%. Depending on the strain, these hydrocarbons are either C_{30} to C_{37} alkenes or C_{23} to C_{33} odd numbered alkenes [62]. These hydrocarbons are mainly accumulated on the outside of the cell, making extraction easier than when the cell wall has to be passed to reach the organics inside the cell. *B. braunii* lives in freshwater, but can also adapt to large range of (sea) salt concentrations. *B. braunii*'s main disadvantage is that it grows very slowly (doubling time is 72 h) [27]. This is >20 times slower than fast-growing algae, therefore only low-investment growth systems like raceway ponds are interesting [63].

Lipids and biodiesel. Lipids (trigelicerides, isoprenoids, phospholipids and glycolipids) are one of

the main components of microalgae. Depending on the species and growth conditions 2–60% of total cell dry matter, as membrane components, storage products, metabolites and storages of energy can be lipid. In comparison with plant oil, algal oil is unsaturated to a larger degree, making it less appropriate for direct combustion in sensitive engines. Triglycerides and free fatty acids can be converted into biodiesel [64]. Triglyceride production rates in algae are 45–220 times higher than terrestrial plants [65]. The global biodiesel market is estimated to reach 37 billion gallons by 2016, growing at an average annual growth of 42%, being Europe the major biodiesel market for the next decade or so, closely followed by US market [66].

Carbohydrates and ethanol. Algal carbohydrates typically are complex mixtures of mono-, poly-, and oligosaccharides, with pentoses and hexoses. Cellulose (aglucan) and glycoproteins are in the cell walls. Algae species starch contents can be over 50%. With new technologies, cellulose and hemicellulose can be hydrolysed to sugars [67], creating the possibility of converting an even larger part of algal dry matter to ethanol. Algae have some beneficial characteristics compared to woody biomass. Most notable is the absence of lignin in algae. Furthermore, algae composition is generally much more uniform and consistent than biomass from terrestrial plants, because algae lack specific functional parts such as roots and leaves. Other algal species, with high starch contents (9 and 69%) [4] are promising feedstock for ethanol production. Currently bioethanol is produced by fermenting sugars, which in the case of corn are derived from hydrolyzing starch. It is estimated that approximately 5000–15.000 gallons of ethanol/acre/ year can be produced by algae which is 10 to 30 times higher than corn starch ethanol systems (400–500 gallons of ethanol/acre/year) [68]. Bioethanol can be used as a biofuel which can replace part of the fossil-derived petrol. Butanol is another kind of alcohol that can be produced by algae. The butanol fermentation process was first commercialised in the UK in 1916. However, it was largely abandoned in the 1950s because it was cheaper to derive butanol from mineral oil. As an automotive fuel, butanol has a number of advantages over ethanol, including lower vapor pressure resulting in fewer evaporative emissions, the ability to be distributed via pipeline as opposed to truck or train, and a higher energy density. Because biobutanol can be produced using the same feedstocks as ethanol, and with a very similar production process, the final product separation process (distillation and dehydration) would be problematical [3].

Hydrogen. Hydrogen is an important fuel with wide applications in fuel cells, liquefaction of coal, and upgrading of heavy oils. Different production process of hydrogen from biomass was described in Fig. 2. Hydrogen can be produced by dark and photo fermentation of organic materials and photolysis of water by special microalgal species [69]. Hydrogen can be applied in mobile applications with only water as exhaust product and no NOx emissions when used in a fuel cell. Currently, hydrogen gas is produced by steam reformation of fossil fuels. Biohydrogen production from microalgae has been known for more than 65 years and was first observed in the green alga *Scenedesmus obliquus* and later identified in many other photosynthetic species such as cyanobacteria [70]. Most studies on algal hydrogen production have been carried out using the green alga *Chlamydomonas reinhardtii*. A major advantage of hydrogen production is that hydrogen does not accumulate in the culture and quickly released into the gas phase not to be at levels toxic to the cells [71]. Efforts to improve biohydrogen production using photosynthetic bacteria and algae mainly rely on engineering of two enzymes, nitrogenase and hydrogenase, which evolve H_2 during catalysis [72]. Biological hydrogen production is also performed in two stages of different atmospheric conditions, the first stage for cell growth followed by the second stage for hydrogen evolution. Nitrogen starvation is often used at the end of the growth stage as an efficient metabolic stress to induce the activity of nitrogenase. A nitrogen-free gas phase such as argon plus carbon dioxide gives a high hydrogen evolution rate [73]. Other algal species such as *Chlorococcum littorale* and *Platymonas subcordiformis* are also investigated for hydrogen evolution [74]. Some algae can make hydrogen directly from sunlight and water, although only in the complete absence of oxygen [75]. Direct biophotolysis is done by green algae and can produce H_2 directly from water and require high intensity of light and O_2 can be poisonous to the system. In direct biophotolysis sunlight and organisms containing either hydrogenase or nitrogenase and closed photobioreactor are used. Unlike nitrogenase, hydrogenase is not cut up within the cell, a low partial pressure of oxygen must be maintained, either by in situ removal or a sweeping gas. Indirect biophotolysis performs by the normal oxygenic photosynthetic processes of microalgae to produce carbohydrate before producing hydrogen through dark anaerobic photosynthetic mechanisms [76]. The use of carbohydrates as intermediates separates the production of oxygen and hydrogen, so avoiding an obstacle of direct photolysis. This can provide spatial separation that lowers costs. The second, dark anaerobic stage is essentially to convert carbohydrate into hydrogen, carbon dioxide and organic acids. In the third stage an oxygenic photosynthetic mechanisms are used to produce hydrogen. Indirect biophotolysis processes are the paths followed by cyanobacteria. Because of the high rates of H_2 production, *Anabaena* species have been subjected to intense study. Hydrogen production has also been investigated by other species, including *Nostoc muscorum*, *N. spongiaeforme*, *Westiellopsis prolifica*, *Oscillotoria Miami* BG7, *Aphanothece halophytico* [77, 78]. Studies indicate that maximum yield for hydrogen production through green-algae of about 98 kg H_2 ha^{-1} day^{-1} [79].

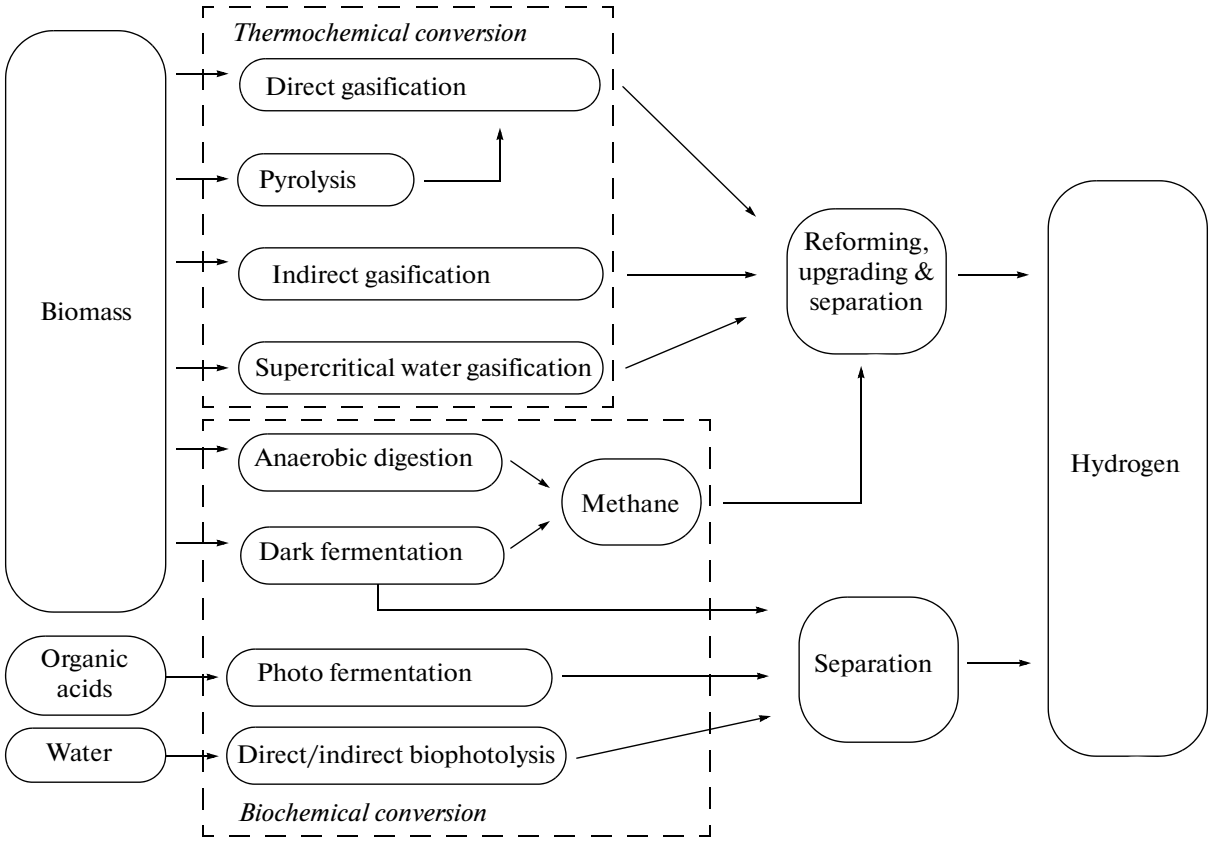

Fig. 2. Hydrogen pathways from biomass and biochemistry [82].

Biogas/biomethane. In biochemical conversion which breaks down sugars using enzymatic anaerobic digestion is more famouse. It can produce a mixture of methane and carbon dioxide with small amounts of hydrogen, hydrogen sulphide and ammonia.

Algae can be digested by bacteria in anaerobic digesters. To optimize biogas yields from anaerobic digestion, the carbon/nitrogen (C/N) balance in the feedstock should be in a range of 25–30. The technical feasibility of anaerobic digestion will also depend on the size of the operation (a minimum of around 150 dry t of biomass per year is required to feed a very small digester), and possibly the availability of additional high-carbon feedstocks, such as waste paper [80]. It has to be mentioned that biomethane production from microalgae currently is not competitive with biomethane production from maize or other crops because the production of biomass is expensive and production capacity is far too low today to feed the demand of commercial biogas plants [53]. The anaerobic digestion of algae can achieve methane yields of about 250 $m^3 t^{-1}$ of algae [81]. There is other ways to produce methane from biomass. The off-gas from the FT reactor is used for bio-gas production through methanation. During methanation, CO and CO_2 react with H_2 to produce methane (CH_4) and water. Another pathway for bio-gas production is biomass gasification in supercritical water, but the composition of the syngas makes this route less favourable for bio-gas production. Depending on the gasification temperature, gasification in supercritical water is more suitable for hydrogen production [82].

Bio-oil and bio-syngas. When biomass is processed under high temperature at the absence of oxygen, products are produced as three phases: the vapor phase, the liquid phase, and the solid phase. The liquid phase is a complex mixture called bio-oil. Up-grading of the product including hydrodeoxygenation and Zeolite upgrading, both converting the bio-oil into a fuel which can be used directly in diesel engines. Bio-oils can also be blended into diesel fuels using expensive surfactants, thus reducing undesirable viscosity characteristics [65].

Gasification processes provide the opportunity to convert renewable biomass feedstocks into clean fuel gases or synthesis gases. The synthesis gas includes is principally CO, H_2, methane, and lighter hydrocarbons, H_2O, PM, tar, alkali vapors, nitrogen and sulfur compounds, and depending on the process used, can contain significant amounts of CO_2 and N_2, the latter mostly from air. High temperature (>1200°C) oxygen-gasification processes produce syngas with very low concentrations of hydrocarbons and higher concen-

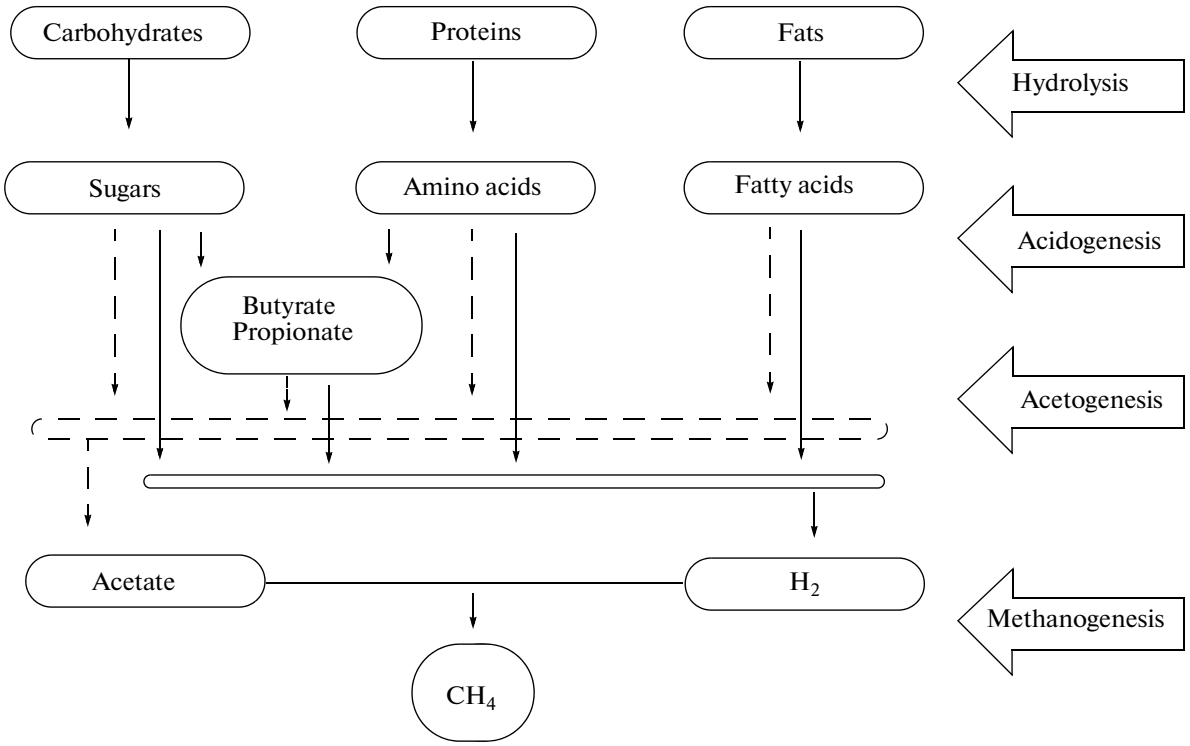

Fig. 3. Degradation steps of anaerobic digestion process [85].

trations of CO and H_2. It is possible to produce diesel fuel from bio-syngas by Fisher-Tropsch synthesis (FTS). The FTS-based gas to liquids technology includes three processing steps, namely syngas generation, syngas conversion, and hydroprocessing. The current commercial applications of the FT process are geared to the production of the valuable linear alpha olefins and of fuels such as liquefied petroleum gas (LPG), gasoline, kerosene, and diesel. Biomass can be converted to bio-syngas by noncatalytic, catalytic, and steam gasification processes [84] (Figs. 3, 4).

Dimethyl ether (DME). DME (CH_3OCH_3) is a synthetic fuel derived from coal, natural gas, or biomass. DME has traditionally been produced in a two-step process where syngas from coal or natural gas was converted into methanol followed by its dehydration [86]. DME offers several advantages over conventional diesel and other transportation fuels. It can be used directly in diesel engines where it produces lower NO_x and SO_x emissions than conventional diesel. The disadvantages of DME are due to its physical properties. The relatively low viscosity causes leaking in pumps and fuel injectors [83].

CONVERSION PATHWAYS FOR BIOFUEL

When biomass is pretreated thermochemically it produces intermediate products bio-oil and residue. Bio-oil must therefore be converted to biofuel under different conditions. Residue can be biochemically or thermochemically converted to a gaseous fuel or a solid, nutrient-rich bioproduct. Conversion pathways include transesterification (chemical, enzymatic), biochemical conversion (fermentation, anaerobic digestion), thermochemical conversion (gasification, pyrolysis, liquefaction) and hydroprocessing. Pyrolysis, gasification, and hydrocracking (use of high-pressure, high-temperature catalysts and hydrogen to produce hydrocarbons) hold promise for creating biomass-based petroleum equivalents, i.e., biocrude, biogasoline, and biodiesel fuels that would be virtually indistinguishable from, and even have advantages over, their petroleum-based counterparts [85].

Transesterification. Transesterification (also called alcoholysis) is the reaction of a fat or oil with an alcohol to form esters and glycerol. There is nothing unique about the transesterification of algal oil compared with that of conventional vegetable oils. Feedstocks obtained from oil pressing (e.g., screw or hydraulic presses) and extraction (e.g., hexane) should be degummed by treating the oil for 4 to 8 h with 300 to 3000 ppm phosphoric acid (depending on the natural levels of gums present) followed by washing with water. Oils can be converted via acid-catalyzed, alkali- or base-catalyzed, or enzymatic transesterification. Alcohols that can be used in the transesterification process are methanol, ethanol, propanol, butanol and amyl alcohol. Methanol and ethanol are used most frequently, especially methanol because of its low cost and its physical and chemical advantages (polar and

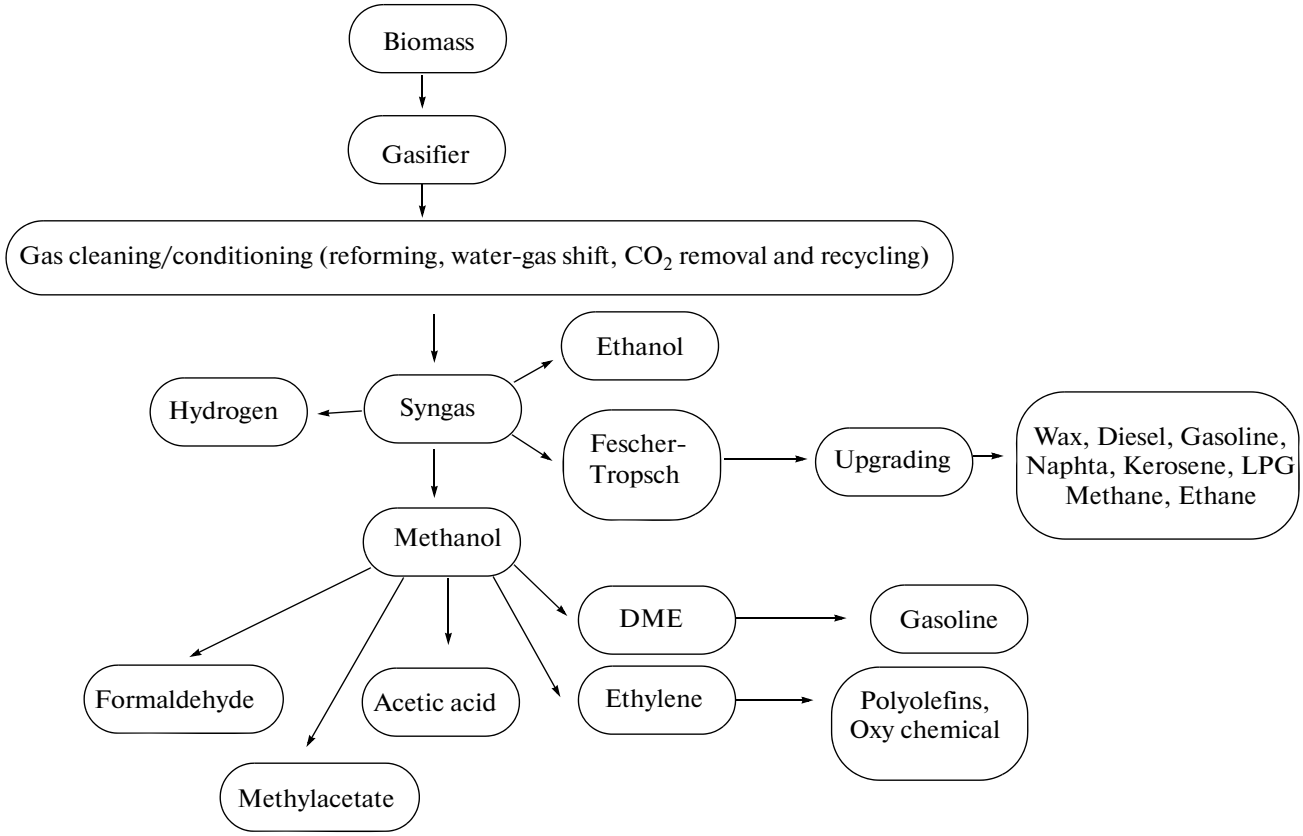

Fig. 4. Syngas products [86].

shortest chain alcohol). This reaction can be catalyzed by alkalis, acids, or enzymes. The alkalis include NaOH, KOH, carbonates and corresponding sodium and potassium alkoxides such as sodium methoxide, sodium ethoxide, sodium propoxide and sodiumbutoxide. Sulfuric acid, sulfonic acids and hydrochloric acid are usually used as acid catalysts. Lipases also can be used as biocatalysts. Alkali-catalyzed transesterification is much faster than acid-catalyzed transesterification and is most often used commercially. An acid catalyst is used when the oil has high acid value.Transesterification can be performed continuously or using a batch process. The main byproducts of transesterification are fatty acid methyl ester (FAME) or fatty acid ethyl ester and glycerol. During conversion, glycerol is periodically or continuously removed from the reaction solution in order to drive the equilibrium reaction toward completion [14]. After transesterification of triglycerides, the products are a mixture of esters, glycerol, alcohol, catalyst and tri-, di- and monoglycerides. Obtaining pure esters is not easy, since there are impurities in the esters, such as di- and monoglycerides. The most important parameters that influence the transesterification reaction are: reaction temperature, ratio of alcohol to vegetable oil, type of acyl donor and acceptor, type and amount of catalyst, mixing intensity, quality (purity, free fatty acid content) of starting materials and water content [5]. Biodiesel is recovered by repeated washing with water to remove glycerol and methanol. Use of lipases offers important advantages, but is not currently feasible because of the relatively high cost of the catalyst [87]. The recovery of glycerol is easy, and purification of the FAME is usually not required. Additionally, the separation of the product and enzyme is facilitated. Lipases obtained from: *Rhizomucor miehei, Rhizopus oryzae, Candida antarctica, Candida rugosa, Pseudomonas cepacia* and *Thermomyces lanuginosa*, but the commercial immobilized lipase from *C. antarctica* (Novozym 435) is the most commonly used enzyme. Instead of using methanol, the lipase-catalyzed synthesis of FAME can also be performed using alternative alcohol donors such as methyl (alkyl) acetate or dimethyl carbonate. The process of such a biodiesel synthesis is irreversible because the intermediate compound (carbonic acid monoacyl ester) immediately decomposes to carbon dioxide and an alcohol [88]. In this process triacetin, instead of glycerol, is produced [89, 90] which can be applied in the synthesis of alkyl esters. Improvement of the enzymatic synthesis of biodiesel can be achieved through the application of *tert*-butanol as a solvent for enzymatic reaction or washing with a solvent for enzyme regeneration [91]. The world's first large-scale biodiesel plant using enzyme technology is operating in Chi-

na with a capacity of 20000 metric t per year using *tert*-butanol as co-solvent, which protects the enzyme and enables very high productivity [92]. For the production of biodiesel using microalgae feedstocks, transesterification can be done in 3 ways: conventional [93, 94], supercritical [84] and in situ [95]. Although the conventional and supercritical transesterification routes involve prior extraction of the microalgae oil from the biomass before the biodiesel production process, the in situ method facilitates the transesterification of the microalgae lipids directly from the biomass, without the need for initial stripping. Lepage and Roy [96] proposed the direct transesterification of human milk and adipose tissue without prior extraction or purification for improved recovery of fatty acids. This reaction was done with alcohol (e.g., methanol) and acid catalyst (e.g., acetyl chloride) followed with heating at 100°C for 1 h under sealed cap. Rodriguez-Ruiz et al. [97] applied this method to microalgal biomass and modified the approach to include hexane in the reaction phase in order to avoid a final purification step. Moreover, Rodriguez-Ruiz and coworkers found that the entire reaction could be shortened to 10 min. Finally, Carvalho and Malcata [98] found that when applying direct transesterification using an acid catalyst (i.e. acetyl chloride), the efficiency of the reaction is increased when a second less polar solvent such as diethyl ether or toluene was mixed with the methanol to modify the polarity of the reaction medium. Supercritical transesterification approach (simultaneous extraction/transesterification) can also be applied for algal oil extracts. Extraction is efficient at the modest operating temperatures, for example, at less than 50°C, thus ensuring maximum product stability and quality. Additionally, supercritical fluids can be used on whole algae without dewatering, thereby increasing the efficiency of the process.

Biochemical fermentation/anaerobic digestion. Anaerobic fermentation is the more common biochemical approach to convert algal biomass to butanol, methanol, acetone and also converts residue to biogas for biofuel. Byproducts of the fermentation process are CO_2, methane, water, and several acids, including acetic and lactic acids. Methane is suitable for electricity and heat; whereas other liquid byproducts, such as acetone, are suitable for recycling as eutrophied process water. Anaerobic digestion is not currently a popular pathway for algal residue conversion. The three main bottlenecks to digest microalgae are [99]:

– low biodegradability of microalgae depending on both the biochemical composition and the nature of the cell wall;

– high cellular protein content results in ammonia release which can lead to potential toxicity;

– presence of sodium for marine species can also affect the digester performance.

When the cell lipid content does not exceed 40%, anaerobic digestion of the whole biomass appears to be the optimal strategy on an energy balance basis, for the energetic recovery of cell biomass [99]. Methane fermentation is the consequence of a series of metabolic interactions among various groups of microorganisms. The first group of microorganisms secretes enzymes which hydrolyze polymeric materials to monomers such as glucose and amino acids, which are subsequently converted to higher volatile fatty acids, H_2 and acetic acid. In the second stage, hydrogen-producing acetogenic bacteria convert the higher volatile fatty acids like propionic and butyric acids to H_2, CO_2, and acetic acid. Finally, the third group, methanogenic bacteria convert H_2, CO_2, and acetate, to CH_4 and CO_2 [100].

Thermochemical conversion. Endothermochemical conversion involves the consumption of energy to convert a fuel source (biomass) into a different chemical state (oil and residue). Exothermochemical conversion (releasing energy) via combustion to generate power that is not within the scope of this review. The thermochemical conversion processes involve heating of biomass at high temperatures. There are two basic approaches. The first is combustion and gasification of biomass and its conversion to hydrocarbons. The second approach is to liquefy biomass directly by high-temperature pyrolysis, high-pressure liquefaction and ultra-pyrolysis [3].

Combustion. Combustion is the burning of biomass in air. It converts the chemical energy stored in the biomass into heat, mechanical power, or electricity using different process equipment. Combustion produces hot gases at temperatures around 800 to 1000°C. This is an older method of utilizing biomass for obtaining energy [3].

Gasification. Gasification is a thermochemical process that, in the near absence of oxygen, converts organic material into a combustible gas called synthesis gas (syngas). Syngas, comprised of mainly of CO, CO_2, H_2, CH_4, water and tar vapors (long-chain aliphatics), and ash particles, contains 70–80% of the energy originally present in the biomass feedstock. With proprietary catalysts, syngas yields fuel gases H_2, ethanol, methanol, and DME. Gas components can be used in turbines and boilers or as feed gas for the production of liquid alkanes by Fischer-Tropsch (FT) synthesis. Gasification processes can be classified into two categories: conventional gasification and supercritical water gasification. Conventional gasification is to decompose dry biomass at high temperature (800–1000°C or higher), pressure, and the absence of oxygen to the materials, which are further decomposed into small molecular combustible gas, usually with the help of gasification catalysts. Conventional gasification also requires dry biomass with moisture content not higher than 15–20%. Supercritical water gasification, on the other hand, relies on the existence of supercritical water to cause the hydrolysis of biomass components to produce smaller molecules [101],

whereas supercritical water (hydrothermal) gasification occurs at 347°C with a metal catalyst or at 697°C with a carbonaceous or alkali catalyst. Due to the different reaction mechanisms, supercritical water gasification owns some unique advantages over conventional gasification. Firstly, supercritical water gasification is suitable for recovery of energy from wet biomass, avoiding the energy-intensive drying process. Secondly, supercritical water has some specific features such as high solubility of biomass components and products, which could achieve homogeneous reaction and allows simple separation of the gas products from liquid phase at the end of the reaction [101]. Thirdly, supercritical water gasification requires more moderate conditions than that of conventional gasification. The most significant problem with FTS in gasification is the cost of clean-up and tar reforming. Tars are high molecular weight molecules that can develop during the gasification process. The tars must be removed because they cause coking of the synthesis catalyst and any other catalysts used in the syngas cleanup process. Tar formation can be minimized or avoided via entrained-flow gasification at high temperatures [102]. While this technology requires sub-millimeter sized particles, algae may have a unique advantage in this process. Reforming is a process in which biomass is gasified in the presence of another reactant such as steam, steam-oxygen or steam-air. Gases with low calorific value are generally formed when there is direct contact with air, as this results in dilution with nitrogen. Medium energy gases result when oxygen is used or when air is used but the gasifier is heated indirectly. High-energy gases result at lower temperatures and high pressures which favour the production of methane and other light hydrocarbons [103]. Tars in the product gas are problematic because they condense in exit pipes and on particulate filters leading to blockages and clogged filters. They also cause clogging of fuel lines and injectors in internal combustion engines. There are currently three basic pathways to overcome the tar-related problems:

• fluidised-bed gasification + catalytic reforming;

• fluidised-bed gasification + solvent-based tar removal;

• entrained-flow gasification at high temperatures.

FT processes form long chain hydrocarbons from catalytic combination of CO and H_2. In case of high temperature gasification (1200°C to 1600°C), it leads to few hydrocarbons in the product gas, and a higher proportion of CO and H_2. If the ratio of H_2 to CO (syngas or biosyngas) is 2 : 1, FT synthesis could be an option to convert syngas into high quality synthetic biofuels which are fully compatible with conventional fossil fuel engines. FT processes use catalysts based on iron, cobalt, ruthenium, and potassium, and have been extensively characterized, operate at high pressures (2.50–4.50 MPa) and temperatures (between 220°C and 450°C). The process can co-produce electricity, heat, and a liquid fuel. However, the multistage process requires high capital cost resulting in considerably high cost of biofuels thus making the process economically unviable [3].

Pyrolysis. Pyrolysis, also known as thermal cracking, is a thermochemical process to convert dry biomass to liquid (termed bio-oil or bio-crude), solid, and gaseous fractions by heating the biomass in absence of oxygen with the aid of a catalyst. It involves heating in the absence of air or oxygen and cleavage of chemical bonds to yield small molecules. Pyrolytic chemistry is difficult to characterize because of the variety of reaction paths and the variety of reaction products that may be obtained from the reactions that occur. This process is applied at temperatures above 430°C (500–600°C, 0.1–0.5 MPa). When the off-gases are cooled, liquids condense to produce oil. This organic oil is often referred to pyrolysis oil or bio-oil. Slow pyrolysis produces a black, tarry oil residue, while fast pyrolysis outputs dark-brown, low viscosity oil. The yields for fast pyrolysis vary extensively from 18 to 80% efficiency. Carbon monoxide, alkanes, alkenes, charcoal, phenol-formaldehyde resins, carboxylic acid and wastewater are common byproducts of pyrolysis. Catalysts have been used in many studies, largely metallic salts, to obtain paraffins and olefins similar to those present in petroleum sources. Pyrolysis can be used to produce predominantly bio-oil if flash pyrolysis is used, enabling the conversion of biomass to bio-crude with an efficiency of up to 80% [104]. Upgrading bio-oils can be achieved by lowering the oxygen content and removing alkalis by means of hydrogenation and catalytic cracking of the oil [105]. Hydrogenation is mainly employed for the production of methane by hydro-gasification. Synthesis gas reacts with hydrogen to yield methane. The shredded biomass may directly be converted with the hydrogen-containing gas to a gas containing relatively high methane concentrations in the first-stage reactor. A few studies have been carried out on the production of fuel oil from microalgae by pyrolysis [106, 107]. Yields of 18 and 24% high-quality bio-oil were obtained by fast pyrolysis of *C. protothecoides* and *Microcystis aeruginosa* at temperature of 500°C [107], which has a potential for commercial application of large-scale production of liquid fuels. It was also noticed [108] that *C. protothecoides* was preferable for pyrolysis over *Spirulina platensis* [109]. The quantity and quality of the pyrolysis products depend on various parameters, such as reaction temperature, pressure, heating rate, reaction time, etc. Lower process temperature and longer vapor residence times favor the production of charcoal, whereas high temperature and long vapor residence time increase the gas yields. Moderate temperature and short vapor residence time are optimum for higher liquid yields [110]. The pyrolysis process may be endothermic, or exothermic, depending on the temperature of the reacting system. The pyrolysis processes can be slow, fast and flash. Slow pyrolysis is a conventional process whereby

the heating rate is kept slow (approximately 5–7°C/min) [111]. This slow heating rate leads to higher char (particulates) yields than the liquid and gaseous products. Fast pyrolysis is considered a better process than conventional, slow pyrolysis. In this, the heating rates are kept high, about 300 to 500°C/min and the liquid product yield is higher. Fluidized-bed reactors are best suited for this process as they offer high heating rates, rapid devolatilization and also are easy to operate. Reactors such as entrained flow reactors, circulating fluidized-bed reactors, rotating reactors, etc. are used for this purpose. This is an improved version of fast pyrolysis, whereby high reaction temperature is obtained within a few seconds. The heating rates are very high, about 1000°C/min with reaction times of few to several seconds. This is carried out at atmospheric pressure. Because there is rapid heating of the biomass, for better yields smaller particle size are favored. Flash pyrolysis can be categorized as.

1. Flash hydro-pyrolysis: at the presence of hydrogen, at 20 MPa.

2. Solar flash pyrolysis: solar energy is used.

3. Rapid thermal process: involves very short residence time of 30 ms to 1.5 s and is carried out at temperatures between 900 and 950°C to eliminate the side reactions in the system, with high yields of the desired product.

4. Vacuum flash pyrolysis: with a vaccum to stop the secondary decomposition reactions, giving higher liquid yields, and reduces gas production because of the quick removal of the liquid from the system.

5. Catalytic biomass pyrolysis: to improve the quality of the oil (the oil from pyrolysis processes is generally unsuitable) [84, 112].

Liquefaction. Liquefaction, a thermochemical pretreatment process that converts organic material to bio-oil at low temperature and high pressure (N_2 at 2–3 MPa to control evaporation) using a catalyst in the presence of hydrogen in just a matter of hours or even minutes. However, it is worth noting that liquefaction is a relatively expensive process due to the use of hydrogen. Conversion is conducted at 300°C, accommodating high moisture content biomass. With the help of a catalyst, the process utilizes the high activity of water in subcritical conditions to decompose biomass materials down to those with a higher energy density or higher value chemicals. Liquefaction can be employed to convert the wet biomass (≥60% moisture) without first reducing the moisture content, thereby avoiding energy-intensive drying of biomass especially algae. The oil product of liquefaction can be treated to yield green diesel or green jet fuel. Process residue can either be burned (i.e., exothermal direct combustion) or converted (e.g., via fermentation) into animal feed or fertilizer. Byproducts include CO_2 and some recalcitrant residue. Bio-oils produced by the thermochemical pretreatment processes of pyrolysis and liquefaction need to be upgraded before they can be used as renewable hydrocarbon biofuels. The mixture is then treated with CH_2Cl_2 catalyst, which causes the separation of biodiesel from an aqueous phase [3].

Cracking. Cracking is a process that breaks ("cracks") the heavier, higher boiling petroleum streams produced by atmospheric or vacuum distillation into lighter molecular weight materials such as gasoline, diesel fuel, jet fuel and kerosene. Often, after cracking, streams may be hydrotreated (reduces nitrogen and aromatic content) or undergo desulfurization. There are two basic types of cracking processes, those using heat and pressure (thermal cracking) to break molecular bonds, and those using a catalyst (catalytic cracking on bio-oil) to facilitate the cracking process. Catalytic cracking is similar to thermal cracking except a catalyst facilitates conversion of the heavier to lighter products and requires less severe operating conditions than thermal cracking. Hydrocracking (on glycerol) is a combination of catalytic cracking and hydrogenation, using high pressure, high temperature, a catalyst, and hydrogen. It is typically used for feedstocks that are difficult to process by either catalytic cracking or reforming. Hydrocracking converts sulfur and nitrogen compounds to hydrogen sulfide and ammonia. Catalytic purification and hydrocracking (breaking apart the triglycerides, otherwise known as splitting) are together known as hydroprocessing. Water, carbon dioxide, and hydrogen are the main process byproducts. Hydrotreating (removing the oxygen, or otherwise known as decarboxylation for aqueous sugar stream) and hydrogenation (saturating the double bonds) can be done through the process. Hydroprocessing includes hydrocracking and hydrotreating. Hydroprocessing (350°C to 450°C and a pressure from 4.8 MPa to about 15.2 MPa and a liquid hourly space velocity of 0.5 to 5.0 per h, depending on feedstock) in the presence of catalysts is used to upgrade a crude, intermediary feedstock to a market-ready fuel product. Both algal oil and bio-oil can be accommodated by hydroprocessing to hydrocarbon biofuel such as green or renewable diesel, jet fuel, gasoline, or other light fuel. The key components of upgrading are catalytic purification (by hydrodeoxygenation, hydrodenitrogenation, hydrodesulfurization, and hydrodemetallization) and hydrogenation through catalytic hydrocracking. Hydroprocessing potentially requires large quantities of water and energy to implement the purification and hydrocracking processes. Hydrocracking is to produce diesel while catalytic cracking to produce gasoline. The reactions are catalyzed by dual-function catalysts: zeolite catalysts for the cracking function (to reduce the oxygen content and improve the thermal stability) and platinum, nickel, or tungsten oxide for the hydrogenation function. The hydrogenation reactions occur because hydrogen is generated as a by-product in the course of catalytic reforming. The three types of catalytic cracking processes are fluid catalytic cracking, moving-bed catalytic cracking, and Thermofor catalytic cracking. The catalytic

cracking process is very flexible, and operating parameters can be adjusted to meet changing product demand. In addition to cracking, catalytic activities include dehydrogenation, hydrogenation, and isomerization in the presence of catalysts such as a crystalline aluminosilicates (zeolites) or molecular sieves. The literature on catalytic cracking of vegetable oils can be grouped into four main catalyst types including: (1) molecular sieve catalysts, (2) activated alumina catalysts, (3) transition metal catalysts and (4) sodium carbonate [112].

QUALITY ISSUES OF BIOFUEL

Vegetable oils currently used for biodiesel are mainly C_{16} and C_{18}. The most important properties of biofuel, cetane number (ignition quality), cold-flow properties, oxidative stability, and iodine value, are determined by the structure of fatty esters, which are part of it [5, 113]. In turn, properties of fatty esters are determined by the characteristics of fatty acids; i.e., carbon chain length and degree of unsaturation, and the alcohol content [113]. Most microalgal oils differ from plant oils because they are rich in polyunsaturated fatty acids with four or more double bonds [40]. This feature limits the algal species that can be used. Chemical formula of biodiesel is C_{14}–C_{24} methyl esters with boiling point of >475°K, flash point of 420–450°K and light to dark yellow. Microalgal oils are mostly composed of four unsaturated fatty acids, namely palmitolleic (16 : 1), oleic (18 : 1), linoleic (18 : 2) and linolenic acid (18 : 3). Saturated fatty acids such as palmitic (16 : 0) and stearic (18 : 0) also present with a small proportion [114]. These components can polymerize into waxy solids, causing filter clogging and injector fouling. A high level of long chained fatty acid esters also increases the viscosity of the biodiesel, leading to more stress on the diesel injection pump and incomplete fuel combustion, as the droplets formed in the cylinder tend to be larger. The proportion of convertable oil in the algae lipid fraction is another, often overlooked problem specific to algae biodiesel. Due to the high proportion of isoprenoids, glycolipids, phospholipids and aromatics in the algae lipid fraction only a small percentage of the extracted lipid fraction might be mono-, di-, and triglycerides suitable for transesterification. Numerous authors report that the fatty acid composition (chain length, saturation) does not only vary significantly between different algae species and genera, but also that the fatty acid composition of algae is affected by environmental factors such as illumination, temperature, nutrient and CO_2 availability, etc. For example, microalgal hydrocarbon content has been shown to be affected by nutrient availability, increasing under nutrient-limited conditions (particularly nitrogen) [28].

Cetane number. Cetane number is an important parameter in evaluating the quality of biodiesel fuel. It is established that the FAME composition of the methyl esters used has a predominant effect on the cetane number (CN) of the biodiesel. From the results obtained, it is evident that CN is affected by the % composition of the FAME in the fuel. A higher cetane number gives better starting properties and a shorter ignition delay (the interval between injection and ignition), which produces smoother combustion and a quieter engine. FAME from vegetable oils are mostly unsaturated. CN is affected by the % composition of FAME, as CN values of the saturated FAME are above 60, while those of unsaturated FAME are below 60 [115].

Cloud point (CP). CP the temperature at which a sample of fuel just shows a cloud or haze of methyl or ethyl ester crystals when it is cooled under standard test conditions.

Cold filter plug point (CFPP). CFPP the temperature at which fuel crystals cause a fuel filter to plug. This test is considered a better indicator than cloud point of low temperature operability.

Pour point (PP). PP the lowest temperature at which a fuel will just flow when tested under standard conditions.

Total acid number. Total acid number titrations are performed for both the feedstock before biodiesel production and after for the determination of total acid number. It is necessary to determine the amount of catalyst needed for transesterification to initially pretreat the feedstock to first accomplish acid-catalyzed esterification before conducting the much faster base-catalyzed transesterification procedure.

Iodine values. Iodine values are useful for determination of the overall degree of saturation of the oil, which is important for viscosity, cloud point, and reactivity characteristics. At lower temperatures the oil becomes solid when saturated, but remains liquid with higher degrees of unsaturation. This is important for biodiesel characteristics where ideally the fuel will remain more liquid at lower temperatures, but remains somewhat stable from oxidation or hydrogenation reactions. Iodine is introduced and reacts with the double bonds within the fatty acid structure. The amount of iodine absorbed in grams per 100 ml of oil determines the iodine value. High degrees of unsaturation may result in irreversible polymerization to plastic-like substances. Iodine values greater than 50 may result in decreased engine life, but give better viscosity characteristics in cooler conditions.

Free and total glycerin. In some standard tests one of the more important quality parameters is the glycerin in the free form and the bonded mono-, di-, or triglycerol form, indicating of an incomplete transesterification process or incomplete washing of the final product. Presence of glycerin in the final fuel may result in the fouling of pumps and filters or separation during storage of the fuel. Free and total fatty acids and fatty acid esters may also be determined. HPLC

methods without the need for derivatization could be employed.

Viscosity. It is a measure of resistance to flow of a liquid due to internal friction of one part of a fluid moving over another, affects the atomization of a fuel upon injection into the combustion chamber and thereby, ultimately, the formation of engine deposits. The higher the viscosity, the greater the tendency of the fuel to cause such problems. The viscosity of transesterified oil, i.e., biodiesel, is about an order of magnitude lower than that of the parent oil [14].

ECONOMICAL OVERVIEW OF ALGAL BIOFUEL

The commercial viability of algae-based biofuels production is ultimately going to depend on economics. The major cost components for algae production is harvesting which include: the cost of drying algae, infrastructure and capital expense of equipments and maintanence, chemicals, electricity and manpower for all the operation. The production cost of algal oil depends on many factors, such as yield of biomass from the culture system, oil content, scale of production systems, and cost of recovering oil from algal biomass. Whether algal oil can be an economic source for biofuel in the future is still highly dependent on the petroleum oil price. Chisti [5] used the following equation to estimate the cost of algal oil where it can be a competitive substitute for petroleum diesel:

$$C_{\text{algal oil}} = 25.9 \times 10^{-3}\, C_{\text{petroleum}},$$

where: $C_{\text{algal oil}}$ is the price of microalgal oil in dollars per gallon and $C_{\text{petroleum}}$ is the price of crude oil in dollars per barrel. This equation assumes that algal oil has roughly 80% of the caloric energy value of crude petroleum. For example, with petroleum priced at \$100/barrel, algal oil should cost no more than \$2.59/gallon in order to be competitive with petroleum diesel. Results from algal biofuels modeling and analysis effort indicate a clear set of economic-driven research and development priorities, which can be summarized as follows.

1. Re-focus research and development activities towards minimizing operations and maintenance costs for algae production systems.

2. Emphasize co-product capture and marketability to maximize revenue generation.

3. Aggressively develop technologies and processes that significantly improve total algae yields, without dramatically increasing costs.

4. Reduce total capital costs, through advanced technology, of algae production and harvesting [116].

The economic model may include more than 50 independent variables supported by detailed engineering specifications, commodity market data, and vendor quotes for equipment costs. The model is based on a generic baseline algae growth system and is not specific to any particular technology. It is recognized that the analysis results could vary depending on the growth architecture selected and assumptions for algae productivity. Operations and maintenance costs include all expenses required to operate the algal biofuels system on an annual basis such as utilities (electricity, water, etc.), CO_2, maintenance of the algae growth system (generally N–P–K), CO_2 distribution, water replenishment due to evaporative losses and labor, and nutrients have the greatest influence on operations costs. Utility costs accounted for more than 1/3 of total operations and maintenance expenses. However, when considering the amount of energy required to transport, handle, and process extremely large volumes of water and biomass material, along with considerable evaporative water losses, it becomes apparent why utilities are a significant cost driver. So, the priorities suggested include:

– algal biofuels growth, harvesting (includes oil extraction), system architectures, and processes should be developed and matured in a way that minimizes the amount of energy (electricity, etc.) and water required for nominal operations;

– technologies should be developed and policies implemented that reduce/eliminate the cost of CO_2 for algal biofuel systems.

– algal biofuel technologies should be designed in such a way that maximizes lifetime/longevity and minimizes annual maintenance requirements.

In co-product capture and marketability triacylglycerols for biofuel production represent a relatively small portion of algae-related revenue opportunities. 50%–80% of the material produced in an algal biofuels system will be something other than oils either meals/solids or nutraceuticals. While nutraceutical content in the baseline algae strain is very small, current market values for these products are extremely high and can have a dramatic impact on overall project economics, although the risk of market saturation and depreciating product values exists when considering large-scale algal biofuels production. It is also worth noting that not all algae strains contain nutraceuticals and may not have the revenue opportunities. Nevertheless, harvesting and oil extraction technologies need to focus on highly efficient separation and capture of all valuable algae materials, while minimizing energy and capital costs. Co-product markets must be rigorously analyzed on a regional, national, and international basis to assess the feasibility of realizing revenue opportunities for meals/solids and nutraceuticals. Develop technologies is straight forward and requires no detailed explanation. If the same unit area can produce 2–3 times the algae, assuming that then total project economics improve. Capital costs for an algal biofuels production system are a major commercial viability concern. Estimates for algae system capital costs vary widely, with ranges of 10 to \$100 per acre installed. The algae growth system, water manage-

ment/harvesting/extraction, and CO_2 delivery infrastructure have the greatest capital cost impact [116].

Economics of biodiesel production. Biodiesel is growing into one of the most essential 'near-market' biofuels since all industrial vehicles are diesel-based. "In the past decade, the biodiesel industry has seen massive growth globally, more than doubling in production every 2 years" [53]. "Markets for low-carbon energy products are likely to be worth at least $500 billion per year by 2050, and perhaps much more" [117]. Open algae cultures are used commercially in the US, Japan, Australia, China, India, Israel and elsewhere. Moreover, Aquaflow Bionomic in New Zealand recently announced the first ever commercial production of biodiesel from sewage pond microalgae [118]. Recovery of oil from microalgal biomass and conversion of oil to biodiesel are not affected by whether the biomass is produced in raceways or photobioreactors. Hence, the cost of producing the biomass is the only relevant factor for a comparative assessment of photobioreactors and raceways for producing microalgal biodiesel. It is estimated that the cost of producing a kg of microalgal biomass is 2.95 and 3.80$ for photobioreactors and raceways, respectively. These estimates assume that carbon dioxide is available at no cost [55]. If the annual biomass production capacity is increased to 10.000 t, the cost of production per kg reduces to roughly 0.47 and 0.60$ for photobioreactors and raceways, respectively, because of economy of scale. Assuming that the biomass contains 30% oil by weight, the cost of biomass for providing a liter of oil would be something like 1.40 and 1.81$ for photobioreactors and raceways, respectively. Oil recovered from the lower-cost biomass produced in photobioreactors is estimated to cost $2.80/l. This assumes that the recovery process contributes 50% to the cost of the final recovered oil. If the price of crude oil rises to $80/barrel, then microalgal oil costing $0.55/l is likely to economically substitute for crude petroleum [5].

Economic viability. The economics of microalgae systems are highly sensitive to the assumptions made about costs and revenues, with the difference between the best and worst case assumptions being over € 600/t of algal biomass. It should also be noted that even with the most favourable assumptions about algae production costs (€ 210/t) and revenues for biofuels (€ 120/t algae) and greenhouse gas (GHG) abatement (€ 50/t algae), the process would still not be economically feasible. Thus, fuel-only algal systems are not plausible, at least not in the foreseeable future and additional revenues are required, either from wastewater treatment or higher value co-products. However, the above suggests that to expand the potential of algal production systems in addition to wastewater treatment and associated fertilizer recovery and production, it is important to identify and generate high volume/high value co-products from microalgae biomass that could provide significant revenue (>€ 100/t algae). High value animal feeds (e.g. high in pigments or omega-3 fatty acids) are plausible, as are industrial biopolymers (polysaccharides). A 50% increase of the current achievable annual productivity to 100 t biomass/ha is a key assumption and a pre-requisite for the economic viability of microalgae-based processes for GHG abatement [5, 118, 119]. If existing algae projects can achieve biodiesel production price targets of less than $1 per gallon, the United States may realize its goal of replacing up to 20% of transport fuels by 2020 by using environmentally and economically sustainable fuels from algae production [119]. The combination of the closed photobioreactor and open pond combines the benefits of the two and has been demonstrated to be effective at a 2-ha scale [120].

Enhancement of economic feasibility of biofuels from microalgae:

– biorefinery: the high-value coproduct strategy;

– design of advanced photobioreactors;

– selection of cost-effective technologies for biomass harvesting and drying.

CONCLUSION AND PERSPECTIVES

Increasing energy demand as well as policy commitments towards global environmental change make action in terms of the provision of clean sustainable energy stringent. Algae have the great potentiality to provide valuable biofuels for heat generation, electricity supply and the transport sector. Yield and cost analyses show that the cultivation of algal biomass solely for the production of biofuels is not cost competitive compared to other biomass sources by almost two orders of magnitude, while the energy balance appears to be poor. As it is difficult to identify breakthrough opportunities for significant yield increases and costs savings, algal biofuels are not likely to be competitive in the foreseeable future, also because competing alternative technologies are making significant (and faster) progress. Current high value products from algae or waste-water treatment would not support sufficient quantities to underpin large-scale development of algae for biofuels production or CO_2-mitigation. Therefore, the current large investments in the production of algal biofuels are highly premature, and divert funds from more beneficial and urgently needed technologies. It goes without saying that microalgae can be put to beneficial uses such as the production of chemicals, feed, food additives, and wastewater treatment.

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