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REVIEW

Algal biofuels

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Abstract The world is facing energy crisis and environmental issues due to the depletion of fossil fuels and increasing CO_2 concentration in the atmosphere. Growing microalgae can contribute to practical solutions for these global problems because they can harvest solar energy and capture CO_2 by converting it into biofuel using photosynthesis. Microalgae are robust organisms capable of rapid growth under a variety of conditions including in open ponds or closed photobioreactors. Their reduced biomass compounds can be used as the feedstock for mass production of a variety of biofuels. As another advantage, their ability to accumulate or secrete biofuels can be controlled by changing their growth conditions or metabolic engineering. This review is aimed to highlight different forms of biofuels produced by microalgae and the approaches taken to improve their biofuel productivity. The costs for industrial-scale production of algal biofuels in open ponds or closed photobioreactors are analyzed. Different strategies for photoproduction of hydrogen by the hydrogenase enzyme of green algae are discussed. Algae are also good sources of biodiesel since some species can make large quantities of lipids as their biomass. The lipid contents for some of the best oil-producing strains of algae in optimized growth conditions are reviewed. The potential of microalgae for producing petroleum related chemicals or ready-make fuels such as bioethanol, triterpenic hydrocarbons, isobutyraldehyde, isobutanol, and isoprene from their biomass are also presented.

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Abbreviations

Chl	Chlorophyll
DMAPP	Dimethylallyl diphosphate
FAMEs	Fatty acid methyl esters
FDX	Ferredoxin
FFA	Free fatty acid
FNR	Ferredoxin-$NADP^+$ reductase enzyme
G3P	Glyceraldehyde-3-phosphate
IPP	Isopentenyl diphosphate
LHC	Light-harvesting complex
MEP	Methyl-erythritol-4-phosphate
P	Primary electron donor
PSI	Photosystem I
PSII	Photosystem II
Q_A	Tightly-bound plastoquinone
Q_B	Mobile plastoquinone
RuBisCO	Ribulose bis-phosphate carboxylase-oxygenase
TAG	Triacylglycerols

Introduction

Viable alternative energy sources are needed as the demand for oil and gas resources increases with the growth of the global economy and population. Algae can offer an alternative renewable source of energy since they use photosynthesis to capture CO_2 by converting it into reduced carbon sources as biofuels. These biofuels are produced from sunlight, CO_2 and water which are all renewable resources. 183 tons of CO_2 can be captured when 100 tons

of microalgal biomass is produced (Chisti 2008). The photosynthesis process can be oxygenic or anoxygenic. Oxygenic organisms, plants, algae, and cyanobacteria, are capable of oxidizing water using it as the ultimate electron donor producing oxygen while anoxygenic organisms, such as green sulfur and purple non-sulfur bacteria, use substrates like H_2S or organic acids as the electron donors (Blankenship and Govindjee 2007).

The photosynthesis process starts as energy of light is absorbed and employed in photochemistry and electron flow which is initiated by special photoactive chlorophyll (Chl) molecules forming the primary electron donors of two membrane-bound photosystems. Oxygenic photosynthesis is supported by photosystem I (PSI) and photosystem II (PSII) protein/pigment complexes. PSI and PSII of green algae have their majority of pigments bound to proteins in the light-harvesting complexes (LHC) (Fig. 1). These pigments include Chl *a*, Chl *b*, carotenes and xanthophylls. On the other hand, cyanobacteria (blue-green algae) are prokaryotes capable of performing oxygenic photosynthesis without having chloroplasts (Kiang et al. 2007). The major light-harvesting complexes of cyanobacteria are phycobilisomes which contain Chl *a* and phycobilins pigments. The LHC function is to capture and transfer light energy to the primary electron donor. The primary electron donors of PSII and PSI are P_{680} (Rabinowitch and Govindjee 1965) and P_{700} (Kok 1957), respectively. PSI and PSII complexes have protein-bound cofactors as well as mobile carriers as their electron acceptors/donors. Upon photoexcitation, both P_{680} and P_{700} are capable of giving an electron to a mobile carrier through a chain of unique acceptor molecules. While P_{700} is a heterodimer made of Chl *a* and Chl *a′* (Ben-Shem et al. 2003), two more Chls are believed to be involved in the excited state of P_{680} (Barber and Archer 2001; Groot et al. 2005; Holzwarth et al. 2006; Loll et al. 2005; Raszewski et al. 2008).

Two transmembrane proteins known as D1 and D2 in PSII, and PsaA and PsaB in PSI provide a path for electron transfer through their bound redox cofactors. The oxidizing power of ${P_{680}}^{+}$ is used by PSII to drive water oxidation by oxidizing Mn ions at the catalysis site of the oxygen-evolving complex. Water oxidation is completed and molecular oxygen is released after four oxidizing equivalents are accumulated in the oxygen-evolving complex (Joliot et al. 1969; Kok et al. 1970). The oxidation of two water molecules results in the formation of two fully reduced mobile plastoquinones (Q_B) on the acceptor side of PSII. These mobile quinones give their electrons to oxidized P_{700} molecules via the Cyt b_6f complex. The internal electron transfer of the Cyt b_6f complex contributes to the generation of proton gradient while electrons get transferred from quinones to Cu-containing plastocyanin proteins. Plastocyanins are mobile electron carriers responsible for reducing the ${P_{700}}^{+}$ molecules of the PSI complexes. Two mobile ferredoxin (FDX) proteins reduced by the PSI complexes give their electrons to the ferredoxin-$NADP^+$ reductase enzyme (FNR) for generation of one NADPH from $NADP^+$. The proton gradient established across the membrane through the electron transfer chain powers the ATP synthase which synthesizes ATP from ADP and P_i. Both NADPH and ATP are then used in the Calvin cycle for making carbohydrates from CO_2 (Fig. 1). The ribulose bis-phosphate carboxylase-oxygenase (RuBisCO) enzyme carries out the first key step of the

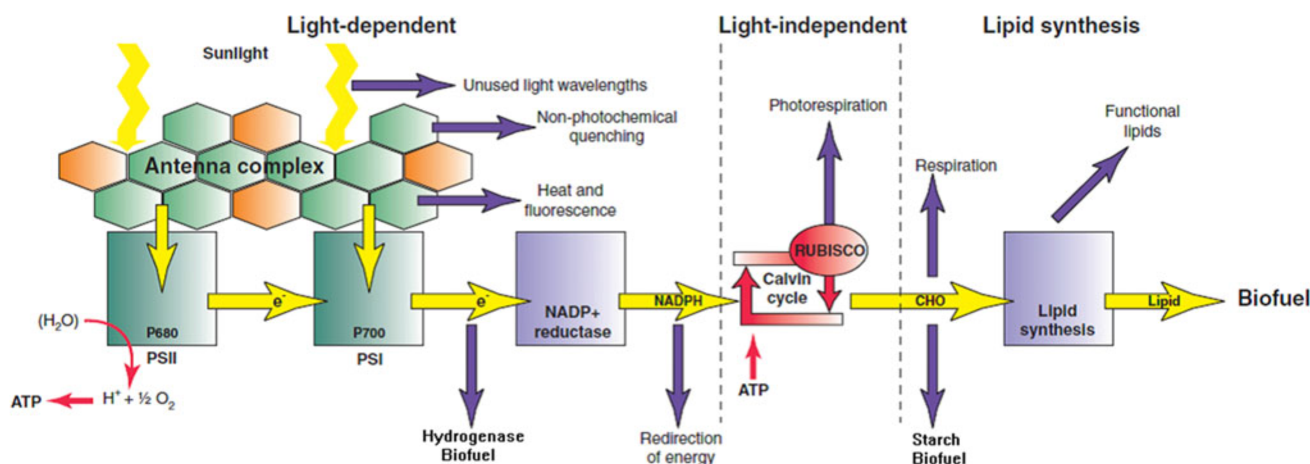

Fig. 1 Photosynthetic solar energy conversion to biofuel. The first part shows the light absorption by photosystem I and II using their antenna complex (LHC), and reactions resulting in the formation of reduced $NADP^+$ and a transmembrane proton gradient. The proton gradient drives the formation of ATP. NAPDH and ATP are used in the Calvin cycle to fix CO_2 into carbohydrates represented by their aldehyde group (CHO) using the RuBisCO enzyme. One fate of fixed carbohydrates is their conversion into lipids as triacylglycerides (TAGs) which can be extracted and processed to produce biodiesel. Adapted with Elsevier permission from Stephenson et al. (2011)

Calvin cycle by binding and reacting CO_2 with ribulose bis-phosphate forming 3-phosphoglycerate which is then reduced to glyceraldehyde 3-phosphate (G3P) by NADPH. Biomass in the form of sugars, cellulose, lipids and other macromolecules are produced from G3P molecules.

Algae

Algae accumulate lipids and carbohydrates (e.g., starch, components of the cell wall-cellulose) which make them good feedstocks for the production of biofuels such as biodiesel and bioethanol. Algae are well adapted to their environments growing relatively fast with some species doubling in number within hours under optimal growth conditions. Harvesting cycle for microalgae is then very short (less than 10 days) and the harvest can be done multiple times or continuously. This is unlike conventional crop plants which are usually harvested once or twice a year. For most algae, the year-around culturing requires a tropical climate, however, some species such as a new *Chlorella* sp. isolated from arctic sea ice can grow at a wide temperature ranging from 4 to 32 °C (Ahn et al. 2012). Total fatty acid in this species reached 39 % of dry biomass under nitrogen starvation. Biomass compositions of several microalgae are given in Table 1.

Table 1 Biomass composition of several microalgae

Source	Carbohydrates (%)	Proteins (%)	Lipids (%)
Anabaena cylindrical	25–30	43–56	4–7
Chalmydomonas rheinhardii	17	48	21
Chlorella vulgaris	12–17	51–58	14–22
Dunaliella salina	32	57	6
Porphyidium cruentum	40–57	28–39	9–14
Spirulina maxima	13–16	60–71	6–7
Chaetoceros muelleri	11–19	44–65	22–44
Chaetoceros calcitrans	10	58	30
Isochrysis galbana	7–25	30–45	23–30
Chlorella sp.	38–40	12–18	28–32
Chlorella protothecoides[a]	11–15	10–53	15–55
Nannochloropsis sp.	n.a.	n.d.	31–68
Neochloris oleoabundans	n.a.	n.d.	35–54
Schizochytrium sp.	n.a.	n.d.	50–77
Scenedesmus obliquus	10–17	50–56	12–14
Quadricauda de Scenedesmus	–	47	1.9

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n.a. not available

[a] Values are for two types of reactors used and not the range

Microalgae can grow under a variety of conditions including in open ponds or closed photobioreactors (Fig. 2) making them a good choice for the production of biofuels even in non-potable water with high salinity. The most common open ponds are raceway ponds and tanks which are currently used for commercial production of algae. Good examples of these facilities are Cyanotech Corporation in Kailua-Kona, Hawaii and Earthrise Nutritionals, California which produce *Spirulina* algae for their nutritional values. The worldwide production of *Spirulina* and *Chlorella* was 5,000 tons year^{-1} in 2004 (Pulz and Gross 2004). Annual production of *Chlorella* and *Spirulina* can reach up to 70 and 13 tons dry cell weight ha^{-1} year^{-1}, respectively (Lee 1997). This study also named commercial producers of microalgae in the Asia Pacific who reported that more than 2,600 tons of *Spirulina* were grown on ~200 ha in China in 1996. A list of several companies producing biofuels from algae can be found in (Singh and Cu 2010).

Open ponds can be constructed and operated easily but their commercial use is somewhat limited to algae that grow under extreme conditions of pH or salinity due to the risk of contamination. *Chaetoceros grafilis* and *Tetraselmis tetrathele* are some examples of algae which can grow in saline water (Araujo et al. 2011). Examples of some microalgae that can live in acidic environments are: *Dunaliella acidophila*, *Chlamydomonas acidophila*, *Chlorella saccharophila, Chlorella protococcoides, Pseudococcomyxa simplex, Stichococcus bacillaris*, and *Viridiella fridericiana* (Gross 2000; Oren 2010). Algae and cyanobacteria such as *Spirulina platensis* can also grow in saline alkaline environments (Gimmler and Degenhard 2001). Some *Scenedesmus* and *Chlorella* species found in limestone mineral hot springs can tolerate high levels of CO_2, while keeping their biomass productivity and carbon fixation activity, making them suitable for capturing CO_2 emitted from industrial processes (Chiu et al. 2008; Westerhoff et al. 2010; Yoo et al. 2010). These thermophilic microalgae can be grown at a facility adjacent to the stacks since they can grow at elevated temperatures with an optimal growth temperature of around 60 °C (Eriksen et al. 1996).

Even though mass production of algae is more cost-effective in open paddlewheel ponds than closed photobioreactors, there is still the problem of contamination and water loss due to evaporation in open ponds. Compared to ponds, the biomass productivities of closed photobioreactors are higher because they provide more control of growth conditions such as light intensity, nutrient levels, flow of gases and temperature (Eriksen 2008; Pulz 2001). Photobioreactors are designed in different configurations to allow for a large illumination surface area while providing sufficient mixing and gas exchange (Kumar et al. 2011). Photobioreactors can be operated in horizontal, vertical, or inclined forms. While sunlight is the light source for the

Fig. 2 Pictures of a raceway pond in Ingrepro, The Netherlands (*left image*) and a low-cost photobioreactor in Proviron, Belgium (*right image*). Reprinted with Elsevier permission from Vandamme et al. (2013)

outdoor photobioreactors, artificial light can also be provided by illuminating the cultures from inside using fiber optics or fluorescent tubes (Barbosa et al. 2005; Matsunaga et al. 1991). These additional light sources can be turned on when there is not enough sunlight reaching the culture.

Closed photobioreactors are made of transparent materials (glass or plastic) to maximize the photosynthetic efficiency (Fig. 3). Tubular photobioreactors are well suited for mass outdoor production of algae due to their large surface area. Mixing in these types of reactors is done by pumps or airlift systems. Algal cells can experience shear stress when growing in raceway ponds, stirred tanks or photobioreactors. *Spirulina, Chlorella* and *Scenedesmus* sp. are examples of some of the species which are tolerant to relatively high levels of shear stress (Pulz and Gross 2004). Good mixing is required to minimize the photoinhibition of algae by exchanging the cells on the outer surface with those in the inner shaded areas. Photosynthesis in microalgae saturates at about 150 μmol quanta m^{-2} s^{-1} which is an order of magnitude lower than sunlight. The excess energy must be dissipated for example through non-photochemical quenching (Fig. 1). Algae grown under saturating lights will synthesize more photoprotective pigments such as β-carotene and zeaxanthin. Photoinhibition is basically the photoinactivation of PSII complexes at high light intensities which is caused by damages made to the D1 subunit (Murata et al. 2007; Polle and Melis 1999).

To decrease the photodamage susceptibility of microalgae and to solve the shading problem in liquid cultures, the expression of light-harvesting antenna was down-regulated (Mussgnug et al. 2007). The minimum number of Chl *a* molecules needed for functional antenna of PSI and PS II were determined to be 95 and 37, respectively (Glick and Melis 1988). Engineered algae with truncated antennae can exhibit an up to threefold improved photosynthetic efficiency and biomass productivity (Melis 2009). In addition, the wild-type algae can be grown in a double-layer photobioreactor with its reduced-pigment mutant. For example, the hydrogen production was increased by 33 % when *Rhodobacter sphaeroides* mutant was grown in the front compartment (Kondo et al. 2002). Using this arrangement, the mutant and wild-type algae were exposed to their optimal high and low light conditions, respectively. The practical maximum efficiency of conversion of sunlight to biomass in oxygenic photosynthesis was calculated to be 8–9 % (Bolton and Hall 1991), while Walker (2009) indicates considering all factors a realistic maximum is 4.5 %. However, only half of this maximum efficiency can be achieved in small-scale cultures of green microalgae and it is further reduced to less than 2 % for large-scale cultures (Melis 2009). For maximum efficiency and productivity, mixing plays a role which helps with the distribution of nutrients and light, gas exchange, and keeping cells in suspension. The productivity of algal biomass may change when cultures are scaled up in large outdoor photobioreactors (Ugwu et al. 2008). A productivity of 0.05 g L^{-1} d^{-1} was obtained for *Haematococcus pluvialis* grown in a 25,000 L parallel tubular photobioreactor or a 55 L bubble column (Lopez et al. 2006; Olaizola 2000). Productivity can also depend on the algae species and strains. For example, productivity of about 2 g L^{-1} d^{-1} was achieved for *Phaeodactylum tricornutum* in a 200 L airlift tubular system (Ugwu et al. 2008).

Biohydrogen

Some green microalgae are capable of producing H_2 by hydrogenase enzymes under anaerobic condition (Fig. 1) (Greenbaum 1982). The anaerobic condition is needed since oxygen inhibits hydrogenase expression and activity (Florin et al. 2001). Filamentous nitrogen fixing cyanobacteria use both nitrogenase and Ni–Fe hydrogenase enzymes for the production of H_2 in their heterocysts (Carrieri et al. 2011; Tiwari and Pandey 2012). The H_2 gas

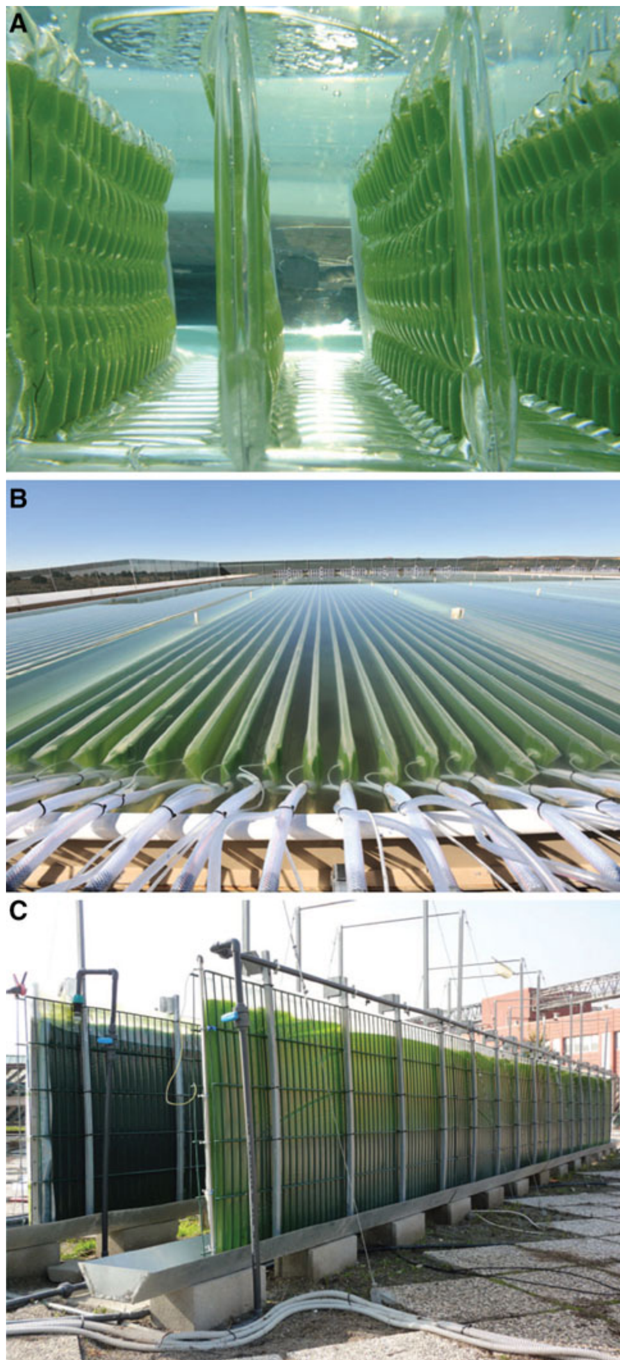

Fig. 3 Examples of low-cost photobioreactors. Flat-panel reactors from **a** Proviron, Belgium **b** Solix Biofuels, USA, and **c** Green wall panel reactors from Fotosintetica & Microbiologica S.r.L., Italy. Reprinted with permission of the American association for the advancement of science from Wijffels and Barbosa (2010)

produced by the nitrogenase enzyme is a byproduct of the reduction reaction of N_2 to NH_3. Electrons needed for reducing protons to form H_2 are provided to these enzymes by reduced ferredoxin proteins. Hydrogen gas production in *Chlamydomonas reinhardtii,* the most studied algae for this purpose, is catalyzed by a bidirectional [Fe–Fe]-hydrogenase enzyme located in the chloroplast. The H-cluster is the site of hydrogenase activity, which contains a binuclear Fe-site coordinated to a [4Fe–4S] cluster through a cysteine (Mulder et al. 2010). The enzyme is sensitive to oxygen because O_2 can react with the [4F–4S] domain of the H-cluster which contains CO and CN ligands (Stripp et al. 2009). The purified hydrogenase enzyme can evolve hydrogen with a specific activity of around 700 µmol H_2 min mg^{-1} of protein (Girbal et al. 2005). Algae is first grown to make substrates needed for the H_2 production during the anaerobic incubation period (Ghirardi et al. 2000).

Sulfur deprivation was shown to create the required conditions for the light-mediated production of H_2 because it decreases the production of O_2 by PSII while increasing accumulation of starch (Melis 2002; Melis et al. 2000). In this method, the photoproduction of H_2 is due to PSI activity supported by direct and indirect biophotolysis processes through water splitting or starch catabolism, respectively (Eroglu and Melis 2011). S-depletion can increase the starch content by eightfold possibly due to the inhibition of cell division and growth (Zhang et al. 2002). The PSII degradation is attributed to the decrease in the D1 protein synthesis. The D1 protein must be continuously repaired since it gets damaged primarily by reactive O_2 species produced by a long-lived excited state of the PSII reaction center, P_{680}, which forms due to restricted transfer of electrons in photochemistry. Also, the electron transfer at the acceptor side between tightly bound plastoquinone (Q_A) and Q_B is affected leading to the formation of reduced Q_A. The H_2 production then allows the reoxidation of Q_A and therefore partial reactivation of PSII. This partial recovery of PSII activity is needed for the H_2 production. PSII-deficient strains of *C. reinhardtii* or cells treated with diuron (DCMU) from the start of S-deprivation do not produce significant amounts of H_2 demonstrating that the PSII activity is required for H_2 production even though active hydrogenase enzymes were synthesized in these cells (Hemschemeier et al. 2008). DCMU inhibits PSII by blocking the electron transfer between Q_A and Q_B. The rapid production of H_2 at this stage is also supported by the reductive power provided by NADPH produced by the metabolism of starch reserves. Under anaerobic conditions, glycolysis becomes the main source of ATP but the excess NADH must be consumed by reducing oxidized glycolytic products to ethanol and succinate. Repeated production of H_2 in the S-deprived culture of *C. reinhardtii* can be achieved by careful re-addition of sulfate into the medium (Kim et al. 2010).

As another approach for creating S-deprivation, *C. reinhardtii* cells with diminished sulfate transport activities were prepared by antisense technology (Chen et al. 2005). These transformants, which were impaired in sulfate

uptake by their chloroplast, photoevolved H_2 like sulfur-deprived cultures. Due to their limited oxygen evolution activity and respiration, the expression of the hydrogenase enzyme in these strains was spontaneous. Increasing H_2 production was also achieved in strains of *C. reinhardtii* containing O_2-tolerant hydrogenases (Flynn et al. 2002; Ghirardi et al. 2005). The strains were generated by random chemical mutagenesis and selected by their abilities to evolve H_2 measured using a chemochromic sensor in the presence of O_2. Various treatments such as adding acetate, changing light conditions or pH, and increasing CO_2 concentration are necessary to sustain the H_2 production. These treatments can affect the photosynthetic electron transport chain and starch accumulation. *C. reinhardtii* cells were grown under two different light qualities, white and red lights. Under red light (692 nm peak, 680–700 nm), PSI activity is favored while PSII activity drops below the respiration level making the culture anaerobic enough for the hydrogenase activity. The H_2 photoproduction under red light was 0.108 mL H_2 mg^{-1} Chl exceeding the amount obtained under white light. Switching between H_2 photoproduction and recovery period was simply achieved by turning the PSI light on or off (Hoshino et al. 2012). Providing acetate to algae co-cultivated with non-sulfur bacteria significantly increased hydrogen production since these photosynthetic bacteria have adapted to absorb near-infrared light not captured by algae (Melis and Melnicki 2006). Hydrogen was produced as a result of dark anaerobic fermentation of the photosynthetic algal biomass by bacteria. H_2 photoproduction was also shown to be improved by preparing a ferredoxin-hydrogenase fusion tested with purified PSI and isolated thylakoids. The specific activity of the fusion enzyme was sixfold higher than the non-fused hydrogenase demonstrating that FNR competes with H_2 production by taking ferredoxin electrons for the NADPH production (Yacoby et al. 2011). Considering these achievements in increasing photobiological production of H_2 by algae in the laboratory, biohydrogen can be considered as a valuable alternative to crop-based biofuel because of its high productivity potential (McKinlay and Harwood 2010).

Biodiesel

Algae can be a good source of biodiesel since some species can make large quantities of lipids as their biomass under certain growth conditions (Table 1). Oils as triacylglycerols (TAG) are high-energy compounds which are overproduced for their reuse during starvation. The main focus has then been to first identify the best oil-producing strains of algae and then optimize the growth conditions to induce the highest possible lipid content (Fig. 4). Microalgae can potentially produce one or two orders of magnitude higher biodiesel than oil palm and cotton (Schenk et al. 2008; Singh et al. 2011). The price of algal oil can change from \$25 to \$2.5 gal^{-1} by increasing productivity to about 10,000 gal $acre^{-1}$ which equals to 50 g m^{-2} day^{-1} at 50 % TAG (Pienkos and Darzins 2009); however, such high productivity may be unobtainable due to photosynthetic efficiency (Walker 2009). There are 60 million ha of land in the USA suitable for growing lipid producing algae such as *Nannochloropsis*, if water is made available, based on hourly historical weather data of many locations in the US and thermal models of industrial-scale outdoor photobioreactor systems (Quinn et al. 2012a). Most of these

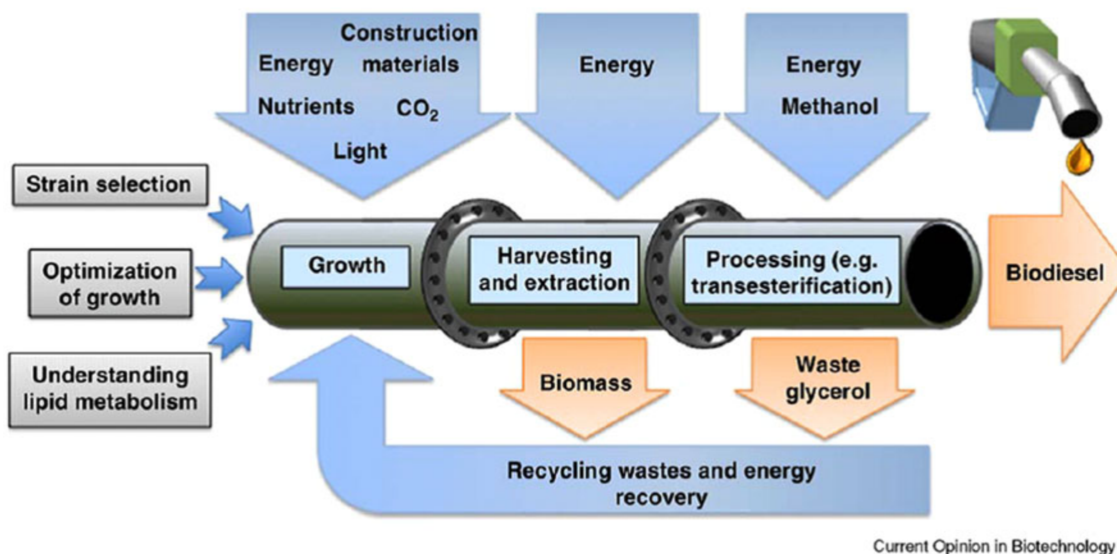

Fig. 4 Algal biodiesel production process starting with strain selection, growth condition optimization and metabolic engineering. Algae are harvested and biodiesel is produced after oil is extracted and transesterified. Reprinted with Elsevier permission from Scott et al. (2010)

available lands are located in Texas, New Mexico, Montana, Arizona, and Nevada.

Even though enough nutrients are initially needed to support the biomass production, nutritional limitation/deprivation can induce lipid synthesis (Rodolfi et al. 2009). Lipid synthesis was induced by nitrogen deprivation in *Nannochloropsis* sp. F&M-M24 in outdoor cultures. The enhanced lipid productivity can be due to the flow of newly fixed carbon atoms diverted from other cellular components (Rodolfi et al. 2009). The flux of the carbon substrates into lipid synthesis is through the formation of acetyl-CoA. Malonyl-CoA, formed from acetyl-CoA by acetyl-CoA carboxylase, is used by the fatty acid synthesis complex for making fatty acids (Merchant et al. 2012). Under N-deprivation, the cellular needs for ATP reduce significantly causing the accumulation of citrate in the mitochondria. The citrate is then transported out of mitochondria into the cytoplasm where is cleaved producing oxaloacetate and acetyl-CoA (Wynn and Ratledge 2005). The conversion of oxaloacetate by malate dehydrogenase into malate and eventually pyruvate by malic enzyme produce NADPH which is needed for fatty acid synthesis in addition to acetyl-CoA. It is the high activity of malic enzyme that makes some species to be oleaginous. On the other hand, TAG and fatty acids are broken down by lipases and enzymes involved in β-oxidation. Interestingly, a 10–20-fold increase in the leaf TAG levels was obtained in *Arabidopsis* plant by down-regulating TAG lipolysis and fatty acid breakdown (Slocombe et al. 2009).

A significant increase in lipid productivity as the result of accumulation of TAGs with saturated and monounsaturated fatty acids was obtained under N-deprivation in nine strains (Breuer et al. 2012). Achieving high lipid content without a significant decrease in the growth requires different level of the N-stress in different species. While high N-stress is needed in some species such as *Neochloris oleoabundans* and *Scenedesmus dimorphus* to increase the lipid productivity, a low level of N-stress was more effective for *Chlorella vulgaris* and *Chlorococcum oleofaciens* (Adams et al. 2013). *Scenedesmus obliquus* and *Chlorella zofingiensis* were found to be the most promising strains for the TAG production since they accumulated TAGs as much as 35 % of their dry weight with a productivity of 250–320 mg L^{-1} day^{-1} (Breuer et al. 2012). The strains retained their biomass productivity after N-depletion. Lipid productivity can also be affected by the CO_2 level. CO_2 levels of 0.5–1 % were shown to improve the lipid contents in *C. vulgaris* (Lv et al. 2010) and *Nannochloropsis salina* (Arudchelvam and Nirmalakhandan 2012). Air with this level of CO_2 concentration can be obtained from an industrial plant.

Biodiesel as fatty acid methyl esters (FAMEs) can be produced by transesterification of TAGs as the major component of algal lipids (Johnson and Wen 2009; Tran et al. 2009). The transesterification of TAGs with alcohol (usually methanol) in the presence of an alkali catalyst (KOH or NaOH) produces fatty acid methyl esters and glycerol. Esterification involves three consecutive reversible reactions of each mole of triglyceride with 3 mol of alcohol supplied in excess. Common organic solvents paired with an alcohol are usually used for extracting the lipids from algae (Sharma et al. 2008; Karmakar et al. 2010), but terpenes as green solvents are also used (Tanzi et al. 2012). Over 80 % of oil can be converted into biodiesel by transesterification. The free fatty acid (FFA) content before esterification with the alkaline catalyst should be less than 2 %. The biodiesel can also be produced using lipase enzymes as the catalyst since they are capable of producing esterified fatty acids from both triglycerides and free fatty acids (Lai et al. 2012; Tran et al. 2012). Immobilized lipase can be used for this process to recycle the enzyme. Two common lipases from *Penicillium expansum* and *Candida antarctica* (Novozym 435) have been used for the production of biofuel in the absence or presence of a cosolvent such as an ionic liquid or *tert*-butanol (Hama and Kondo 2012). At 3:1 molar ratio of methanol to oil, the lipase enzyme converted 98 % of oil to monoalkyl esters of fatty acids in 12 h (Li et al. 2007). Methanol was added stepwise at three different times to avoid inactivation of the lipase enzyme.

Unlike oils, FAMEs are good substitutes for diesel due to their low viscosity. Biodiesel contains biodegradable long-chain alkyl esters with or without double bonds. The length of alkyl chains and degree of unsaturation change the quality of biodiesel by affecting the storage stability and cold-flow properties. A good portion of microalgal lipids are, however, polyunsaturated which means a further hydrogenation step may be needed to reduce the double bonds to lower their susceptibility to oxidation. For example, 50 % of total fatty acids in *S. obliquus* and *C. zofingiensis* is oleic acid (C18:1) while polyunsaturated ones constitute 25–32 % (Breuer et al. 2012). Another important property of biodiesel is cetane number which defines the fuel ignitability. Saturated fatty acids have a much higher cetane number but poor cold-flow properties. This means that ideal biodiesel can be made from a mixture of monounsaturated and saturated fatty acids (Giakoumis 2013; Schenk et al. 2008).

An example of continuing stable cultures of *Nannochloropsis oculata* and *N. salina* algae in large-scale photobioreactors (174,000 L) was presented at a facility operated in Colorado (Quinn et al. 2012b) where the productivity data were collected for over a three year period. The cultures were resistant to contamination over 41 batch transfers mainly due to the filtration of growth media through 0.2 micron filters and salinity (20–27 g L^{-1}). The

photobioreactors were made of polyethylene panels kept in a shallow water basin for thermal and structural support. Mixing and CO_2 were provided through a sparge air system during day light hours (Eriksen et al. 1998). The temperature of the culture was kept close to 25 °C using an evaporative cooling system and a pool heater. Peak lipid productions were 21.1 and 36.3 $m^3\ ha^{-1} year^{-1}$ for *N. oculata* and *N. salina*, respectively.

Other algal biofuels

Microalgal biomass can be used for ethanol production through a fermentation process. Ethanol is produced by the reduction of acetaldehyde generated from pyruvate as a result of anaerobic glycolysis. Fermentation can be done by yeasts, fungi, or bacteria using biomass from broken microalgae cells. *Saccharonmyces cerevisiae* is a commonly used microorganism for this purpose and fermentation is carried out at about 30 °C usually in the presence of a nitrogen source. Distillation can then be applied to purify the ethanol produced by the yeast. *Chorella vulgaris, Spirulina fusiformis*, and *Chlorococum humicola* are good examples of microalga with high carbohydrate content. The carbohydrate is formed as both complex carbohydrates like starch or cellulose and monomeric sugars such as glucose or mannose. Algae contain little or no lignin and hemicellulose than land plants. An important step before beginning the fermentation process is the hydrolysis of starch and cellulose which constitutes the microalgal cell wall (saccharification). Treatment with dilute sulfuric acid at high temperatures (120–160 °C) was shown to be a very effective hydrolysis method (Harun and Danquah 2011; Ho et al. 2012; Miranda et al. 2012). α-Amylase, cellulose and glucoamylase enzymes are also used to release the fermentable sugars from the complex carbohydrates (Choi et al. 2010). *S. cerevisiae* cannot ferment xylose sugar, however, genetically engineered xylose-fermenting strains of this yeast carrying xylose reductase and xylitol dehydrogenase enzymes were developed to improve the biomass conversion to ethanol (Matsushika and Sawayama 2011).

Microalgal metabolic pathways can be manipulated to direct them toward the synthesis of a preferred product by changing the growth environment or metabolic engineering using genetically modified organisms. Genetic engineering was used to create a novel pathway rerouting fixed CO_2 for ethanol synthesis. Two new genes were introduced into *Synechococcus* sp. strain PCC 7942 for encoding pyruvate decarboxylase and alcohol dehydrogenase II enzymes (Deng and Coleman 1999). The synthesized ethanol then diffused from the cells into the culture medium. The pyruvate decarboxylase metabolizes pyruvate to acetaldehyde which is then converted to ethanol by the alcohol dehydrogenase enzyme. Ethanol production in this engineered cyanobacterium occurs during oxygenic photosynthesis with no need for an anaerobic environment. The leftover microalgal biomass residues after lipid extraction can also be hydrolyzed using cellulase, neutrase, and

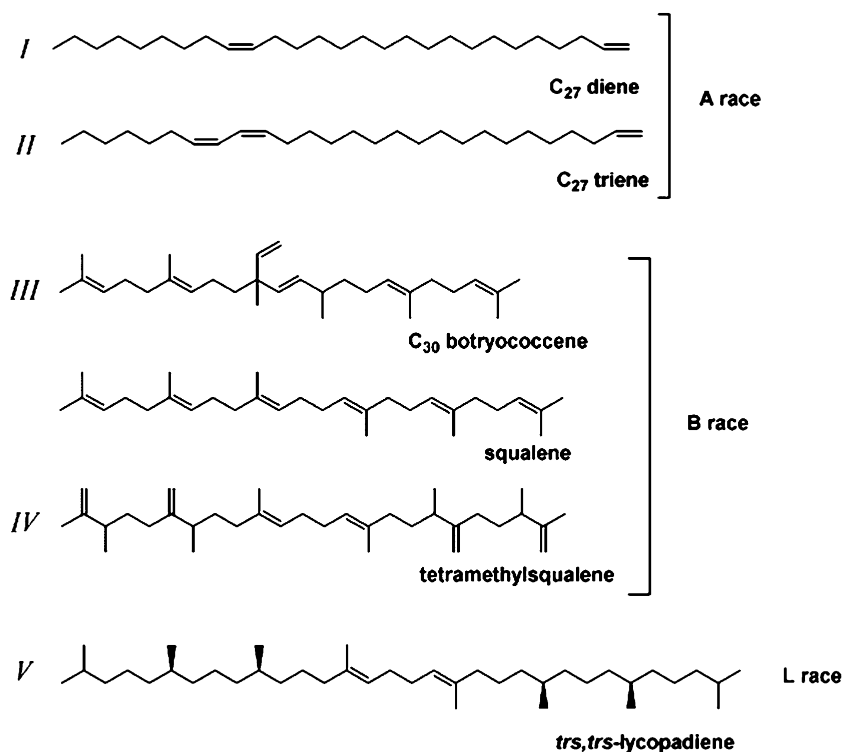

Fig. 5 Different types of hydrocarbons produced by three major races of *B. braunii*. Reprinted with Springer permission from Metzger and Largeau (2005)

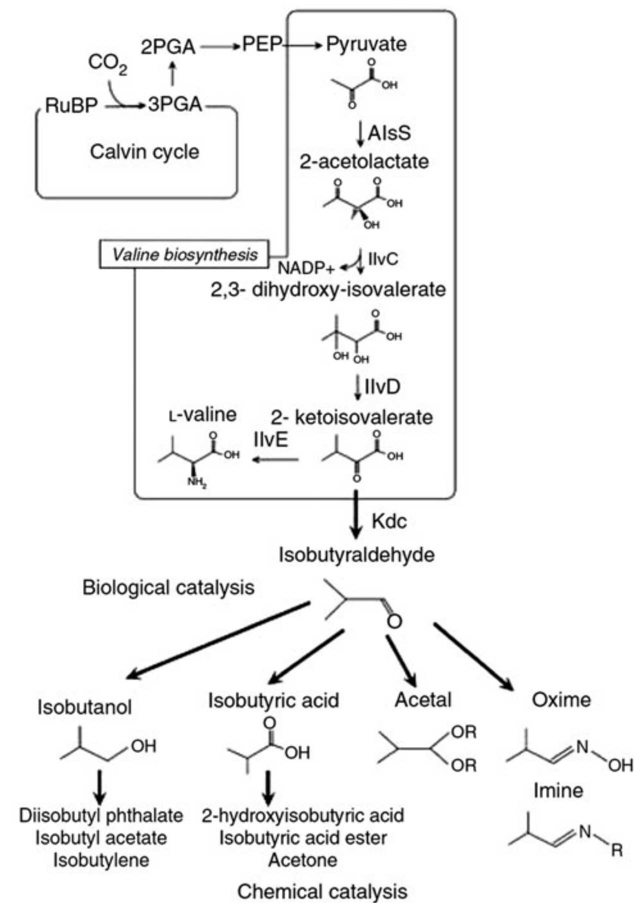

Fig. 6 The production pathway for isobutyraldehyde and isobutanol. Reprinted with Nature Publishing Group permission from Atsumi et al. (2009). Copyright 2009

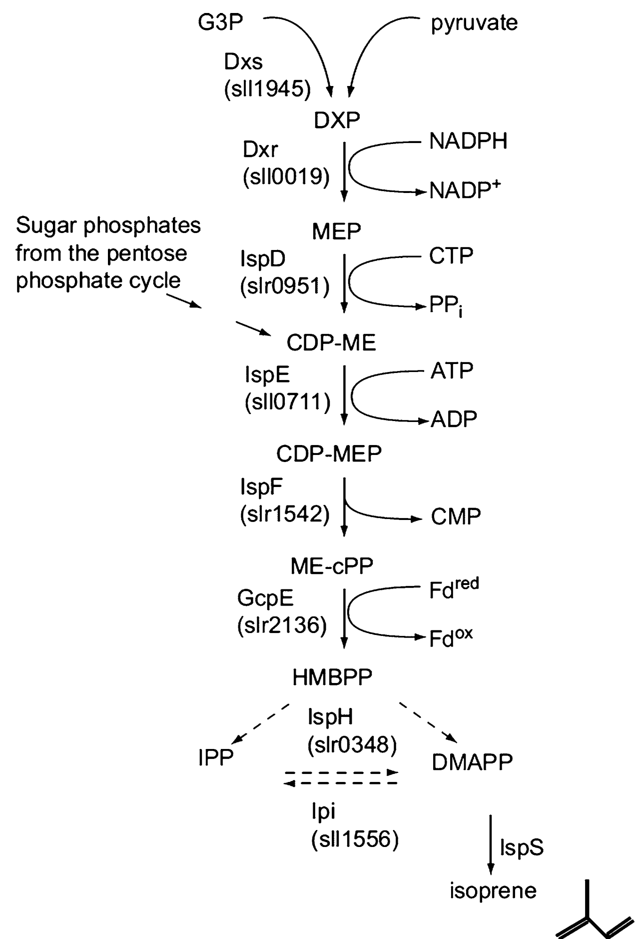

Fig. 7 The production pathway for isoprene using the MEP pathway. Reprinted with Elsevier permission from Lindberg et al. (2010)

alcalase, and fed back to microalgae as a strategy for nutrient recycling (Zheng et al. 2012).

Botryococcus braunii contains high levels of long hydrocarbons which can be converted to shorter hydrocarbons as gasoline, jet fuel or diesel using catalytic cracking (Hillen et al. 1982; Tran et al. 2010). There are three major races of *B. braunii*, each producing different types of hydrocarbons (Metzger and Largeau 2005) (Fig. 5). B-race strains produce triterpenes also called botryococcenes (C_{30}–C_{37}) and methylated squalenes (C_{31}–C_{34}) (Niehaus et al. 2011). These are mainly extracellular hydrocarbons (95 %), found in successive outer walls (Metzger and Largeau 2005), which can be extracted from micro-colonies by organic solvents such as hexane and switchable-polarity solvents (Eroglu and Melis 2010; Frenz et al. 1989; Samori et al. 2010). Business feasibility for the production of triterpenic hydrocarbons from *B. braunii* in abandoned rice fields has been presented (Shiho et al. 2012). The study was based on growing *B. braunii* in a semi-open pond plant in a step by step scaling from smaller size ponds (7.6×10^{-3} and 0.38 ha) to a 20-ha pond. The semi-open ponds were designed as tubular photobioreactors made of transparent plastic membranes to prevent microbial invasion. This production plant could produce fuel at 240 $/barrel with a net CO_2 reduction.

Cyanobacterium *Synechococcus elongatus* PCC 7942 was engineered with a ketoacid decarboxylase gene to produce isobutyraldehyde and isobutanol (Atsumi et al. 2009). The isobutyraldehyde was formed from 2-ketoisovalerate, an intermediate of the valine biosynthesis pathway (Fig. 6). The isobutyraldehyde is then converted to isobutanol in cyanobaterium. To increase the production of isobutyraldehyde, the RuBisCO enzyme was also overexpressed. Long-term production of isobutyraldehyde was achieved since it could readily be collected from growth medium due to its high vapor pressure. Wild-type *S. elongatus* can tolerate high concentrations of isobutyraldehyde up to 750 mg L^{-1}. The engineered cyanobacterium constantly produced about 6 mg $L^{-1}h^{-1}$ of isobutyraldehyde for 9 days.

The cyanobacterium *Synechocystis sp.* PCC6803 was engineered to produce isoprene as a ready-made biofuel

through a methyl-erythritol-4-phosphate (MEP) pathway (Lindberg et al. 2010). As shown in Fig. 7, glyceraldehyde 3-phosphate and pyruvate produced from sugar breakdown are converted into isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) in seven steps in the MEP pathway (Chandran et al. 2011). Isoprenoids produced in the MEP pathway are used by the cell to make a variety of compounds including phytol, β-carotene, lutein and plastoquinone (Wanke et al. 2001). The formation of isoprene was due to the expression of isoprene synthase from *Pueraria montana* in this engineered cyanobacterium. The isoprene synthase enzyme catalyzes the conversion of DMAPP to isoprene. IPP is isomerized to DMAPP by IPP isomerase. Isoprene is a short 5-carbon volatile hydrocarbon which can be easily collected from culture medium. Sealed *Synechocystis* cultures were shown to produce 50 μg isoprene/g dry cell weight/day. Isoprene and 1,3-butadiene are the organic monomers used to make polymerized rubber; however, they are primarily produced by cracking petroleum.

Economics of biofuel

The US ethanol and biodiesel productions are expected to reach 90 and 5 billion liters by 2021, respectively, meeting ~17 % of demand for transport fuel (OECD/FAO 2012). At this time, the major source for the mass production of biofuels is crops. Currently, ~65 % of the vegetable oil production in Europe is used for production of biodiesel, 50 % of Brazilian sugarcane, and 40 % of US corn is used for ethanol production (OECD/FAO 2012). To achieve a high level of biofuel supply as an alternative to fossil fuels, without using farm land and converting food crops to energy crops, other sources of biofuels need to be explored. Production of algae for biofuel can spare the much needed farms for crops since ponds or photobioreactors can be built even on barren lands. The cost of developing algal biofuels for commercialization is currently reduced by government funding opportunities in the area of clean energies. These investments can play a crucial role since the estimated total production cost for about 0.6 tons of microalgae biodiesel (harvested every 12 days) in a 5-ton photobioreactor is about \$100,000 (~\$19 gal^{-1}) for the first year of operation (Lee 2011). However, the estimated cost of algal oil in an open pond system is \$4.75 gal^{-1} (Gallagher 2011). Despite the current high production cost of algal biofuel, it is important that research in this area continues considering the need for energy security, and the environmental benefits of growing algae as a renewable source of energy to mitigate rising atmospheric CO_2 levels.

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