

Microalgae to biofuels: ‘Promising’ alternative and renewable energy, review



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ABSTRACT

The rapid growth of human population has led to mounting energy demands, which is projected to increase by 50% or more by 2030. The natural petroleum can not catch-up the current consumption rate, which is already reported to be 105 times faster than nature can create. Besides, the use of fossil fuels is devastating to our environment through *greenhouse gas* emissions and consequent global warming. Therefore, the search for ‘clean’ energy has become the most overwhelming challenges. Currently, several alternatives are being studied and implemented. Biofuels, fuels from living organisms, provide environmental benefits, since their use leads to a decrease in the harmful emissions of CO₂ and hydrocarbons and, to the elimination of SO_x emissions, with a consequent decrease in the greenhouse effects. Unfortunately, the present biofuel projections are based on feedstocks that are also food commodities and resources suitable for conventional agriculture. One possibility to overcome the problem is the cultivation of *micro-algae* and switching to third generation biofuels, which seem to be a promising source since algae are able to efficiently convert sunlight, water, and CO₂ into a variety of products suitable for renewable energy applications. Therefore, this review is intended to recapitulate current works on micro-algal biofuel production potential and discuss possible ways to put it into practice. This review starts by highlighting the advantages and various forms of micro-algal biofuels. Some of the micro-algal species proved to be suitable for biofuel production so far are considered, with particular emphasis on *Scenedesmus obliquus*. The recent attempts and achievements in improving the economies of production through genetic and metabolic engineering of micro-algal strains are also addressed. Other potential applications such as *wastewater treatment* and *CO₂ mitigation* that can be coupled with biofuel production are described. Finally, the promises and challenges of algae to biofuel industry are uncovered.

1. Introduction

The rapid growth of human population and technological advancements have led to mounting energy demands, which is projected to increase by 50% or more by the year 2030 [1]. The natural petroleum can not compensate the current consumption rate which is already reported to be 105 times faster than nature can provide [2]. Moreover, the use of fossil fuels is devastating to the environment through greenhouse gas emissions and the consequent global warming [3,4]. Rittmann [5] described the danger of depending on fossil fuels from three point of view: depleting fossil-fuel reserves; dwindling resources, leading to geopolitical conflict; and climate change resulting from increasing atmospheric CO₂ concentration. Therefore, the search for ‘clean’ energy has become one of most overwhelming challenges [4,6]. Following this, several alternative sources of energy including solar energy, hydroelectric, geothermal, wind, and biofuels are being studied

and implemented. Of these potential sources of energy, biofuels are seen as real means of achieving the goal of replacing fossil fuels in short term [3].

Biofuels are fuels that contain energy from geologically recent carbon fixation i.e. living organisms. Biofuels can be produced from starch, vegetable oils, animal fats, waste biomass, or algal biomasses, which are non-toxic, biodegradable and renewable [7]. Based on the feedstock types used and their current/future availability, biofuels are categorized into 1st, 2nd, 3rd and 4th generation biofuels [8]. They provide environmental benefits since their use leads to a decrease in the harmful emissions of CO₂, hydrocarbons and particulate matter and to the elimination of SO_x emissions, with consequent reduction of greenhouse effects. In fact, burning biofuels adds less carbon to the environment than burning fossil fuels as the carbon released by burning biofuel already existed as part of the modern carbon cycle [9].

Unfortunately, the present biofuel projections are based on feed-

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stocks that are also food commodities [10] and resources suitable for conventional agriculture [11]. Satyanarayana et al. [12] have pointed out that substitution of diesel by biodiesel involves the use of lands used to produce food and the fiscal incentives by governments are decreasing the lands available for food production.

To this effect, microalgae have attracted much global attention in recent years for the valuable natural products they generate, their ability to remediate effluents and their potential as energy crops [eg. 5]. Algal fuel (oilgae or third-generation biofuel) is a biofuel which is derived from algae. This is the right move for the production of biofuels as algae possess enormous potential (like low-input, high-yield prospect) for renewable energy applications [12–14]. Thus, this potential may enable to completely displace petroleum-derived transport fuels without the controversial argument “food for fuel”. Nowadays, due to increased concern over fossil fuels, energy security, greenhouse gas emissions, and the argument of other biofuels as “food for fuel”, microalgal biofuels attracting more attention [15]. Vieira [16] argued although not yet achieved, the combination of accumulated knowledge from the past, regulatory actions and market needs, unrelenting the quest for commercializing microalgal production. Therefore, the objectives of this review are to recapitulate the attempts undertaken and achievements scored so far on one hand, and to discuss the future promises and challenges in algae-to-biofuel industries, on the other hand, thereby showing research gap.

2. Selection, production and processing of microalgae

Several features of algal physiology are relevant for evaluating their possible incorporation into renewable biofuel applications [3,13,14]. Based on the aforementioned authors, these attributes can be summarized as: i) the solar energy yield with algae could be up to 6–12 times that of terrestrial plants as they are inherently more efficient solar energy converters (3–8% greater than terrestrial); ii) unlike terrestrial, the absence of intractable biopolymers eliminates the need for pre-treatments to breakdown cellular products; iii) their metabolic and ecological diversity allows selection of taxa that are adapted for growth in locally available aquifers or have morphological features that allow cost-effective harvesting and iv) manipulating their end-products through the biosynthetic control of chemical composition by nutrient and environmental stresses.

2.1. Criteria used for the selection of algae strains

Thousands of species of algae have been identified, but certain algal species are more suitable for biofuels use than others. The characteristics of a species should be taken into consideration along with the climate in which the alga will be grown. Properties required of algal species for biofuel production based on the Aquatic Species Program [17] and the findings of other researchers in algal production [18]: lipid (high and constant); growth and efficiency (continuous and stable in fluctuating environments); fouling (minimum); and harvest (easy, flexible and extractable).

Based on the above criteria, many microalgal species have been identified as suitable feedstocks for biofuel production. Each species of microalga produces different ratios of lipids, carbohydrates, and proteins (Table 1). Since the bulk of the natural oil produced by microalgae is in the form of triacylglycerols, which is the right kind of oil for producing biodiesel, microalgae are the exclusive focus in the algae-to-biofuel arena [19]. In order to achieve better economic performance, the lipid content in the microalga need to be raised [20]. Fortunately, many microalgae have the ability to manipulate their metabolism in response to simple manipulations of the chemical composition of their culture medium [3], with consequent high lipid productivity. Some algae, under favorable growth conditions, can double their biomass in less than 24 h [3,21]. Therefore, selection of microalgal species for cultivation should consider species with such

Table 1

Chemical composition of biofuel source microalgae (% of Dry Matter) [6,12,23,24].

Strain	Lipids (%)	Proteins (%)	Carbohydrates (%)
Chlorophyceae			
<i>Chlamydomonas reinhardtii</i>	21	48	17
<i>Chlorella emersonii</i>	29		
<i>Chlorella minutissima</i>	31		
<i>Chlorella protothecoides</i>	55	10–52	10–15
<i>Chlorella pyrenoidosa</i>	2	57	26
<i>Chlorella vulgaris</i>	14–22/56	51–58	12–17
<i>Chlorella sorokiniana</i>	22		
<i>Dunaliella bioculata</i>	8	49	4
<i>Dunaliella primolecta</i>	23		
<i>Dunaliella salina</i>	6	57	32
<i>Dunaliella tertiolecta</i>	28		
<i>Ettlia oleoabundans</i>	35–54		
<i>Botryococcus braunii</i>	25–75		
<i>Haematococcus pluvialis</i>	25		
<i>Monoraphidium minutum</i>	~52		
<i>Scenedesmus dimorphus</i>	16–40	8–18	21–52
<i>Scenedesmus obliquus</i>	35–55	50–56	10–17
<i>Scenedesmus quadricauda</i>	1.9	47	
<i>Spirogyra</i> sp.	11–21	6–20	33–64
<i>Tetraselmis maculata</i>	3	52	15
<i>Tetraselmis suecica</i>	15–23		
Cyanophyceae			
<i>Anabaena cylindrica</i>	4–7	43–56	25–30
<i>Spirulina maxima</i>	6–7	60–71	13–16
<i>Spirulina platensis</i>	4–9	46–63	8–14
<i>Synechococcus</i> sp.	11	63	15
Bacillariophyceae			
<i>Navicula saprophila</i>	~51		
<i>Nitzschia closterium</i>	27		
<i>Phaeodactylum tricornutum</i>	20–30		
<i>Skeletonema costatum</i> (Greville)	21		
<i>Thalassiosira pseudonana</i>	20		
<i>Chaetoceros calcitrans</i>	39	58	10
<i>Chaetoceros muellerii</i>	33	44–65	11–19
Rhodophyceae			
<i>Porphyridium cruentum</i>	9–14	28–39	40–57
Dinophyceae			
<i>Cryptothecodinium cohnii</i>	20		
Euglenophyceae			
<i>Euglena gracilis</i>	4–20	39–61	14–18
Prymnesiophyceae			
<i>Isochrysis galbana</i> Parke	21–38	30–45	7–25
<i>Prymnesium parvum</i>	22–38	28–45	25–33
Labyrinthulomycetes			
<i>Schizochytrium</i> sp.	50–77	n.d.	n.a.

attributes as fast growth, high oil content and easy harvest [22].

Of these, the two most important species one from prokaryotic cyanobacterium, *Microcystis aeruginosa* and another from eukaryotic green microalgae *Scenedesmus obliquus* have been discussed in the next section.

2.1.1. *Microcystis aeruginosa*

Microcystis aeruginosa is a colonial freshwater blue green alga (cyanobacterium) that occurs in surface waters and can form harmful algal blooms [eg. 25]. The cells are organized into colonies which may be seen even with the naked eyes, and usually a spherical shaped, but may lose their coherence to become irregularly shaped over time. The protoplast is light blue-green in color, appearing dark or brown due to optical effects of gas-filled vesicles, which can be useful as a distinguishing characteristic when using light microscopy.

Microcystis aeruginosa have a much simpler lipid and fatty acid composition which can be easily transesterified into biodiesel [Murata and Nishida, 1987 cited in 26,27]. Nevertheless, there are limited research dedication and outputs concerning biodiesel production of this species [26]. As there are blooms of *Microcystis aeruginosa* in many parts of the world, there would have been opportunity to collect

natural bloom and convert it to usable product, biofuel. On top of these, this species is abundant in most of Ethiopian inland waters, with its deleterious effect of releasing toxins to the lake water which is suspected of killing wildlife as reported Amaha Belay and Wood [28], and thus is considered in this review. Even in the oligotrophic Lake Abaya, its common to encounter the high concentration during sample collection by horizontal hauling with plankton net.

Ros et al. [29] reported the biomass productivity of $46.92\% \pm 3.84 \text{ mg L}^{-1} \text{ day}^{-1}$ and lipid content of $28.10 \pm 1.47\%$ in *Microcystis aeruginosa* (the strain reported in the cited paper), isolated from sewage treatment plant and cultured in standard liquid medium. The lipid obtained from this experiment consisted equal proportion of saturated (palmitic and lauric acids) and unsaturated (oleic and linoleic) fatty acids. From these findings they concluded that cyanobacterial lipids from *Microcystis aeruginosa* (strain NPCD-1) have appealing properties for biofuel production. A story of successful mass cultivation of *Microcystis aeruginosa* in open raceway pond at semi-continuous mode of production was reported by Ashokkumar et al. [26], yielding a biomass up to $28 \text{ g m}^{-2} \text{ day}^{-1}$ and lipid content of 21.3%. The lipid extracted was transesterified and produced 90.1% of biodiesel. From this finding, it is also estimated that based on the areal and volumetric biomass productivity inference, *Microcystis aeruginosa* can yield 84.1 t of dry biomass $\text{ha}^{-1} \text{ year}^{-1}$.

The cost of producing biomass of *Microcystis aeruginosa* can be subsidized by the co-production of natural high-value products. For instance, Babu and Wu [30] have determined the synthesis of butylated hydroxytoluene, one of the synthetic antioxidant agents commonly used for food additives to prevent lipid oxidation and food decay, in *Microcystis aeruginosa*. They also reported manipulating the culture condition (i.e. changing light intensity) enhanced magnitudes of butylated hydroxytoluene production, the result confirming that this species constitute a potential source for producing natural butylated hydroxytoluene for large-scale applications. It is obvious that this would also contribute to the cost-effective production of algal biomass intended for biofuel production.

2.1.2. *Scenedesmus obliquus*

Scenedesmus obliquus (Chlorophyta, Chlorococcales, and Scenedesmaceae) is a common cosmopolitan green alga. The thallus is commonly occurring in colonies as multiples of two, with four or eight cells being most common. The morphology of the colony can be varied significantly by varying the medium in which the cells are growing. In a medium with low phosphorus or low salt concentration, *Scenedesmus* is induced to grow as unicellular, forming around $10 \mu\text{m}$ long elliptical cells. It can be easily grown either photoautotrophically in the light, or heterotrophically in the dark, at 30°C [31]. Heterotrophic growth of this alga may be desirable because the cultures need only to be shaken and cells grow within a few days in a special medium. This species was an early investigation object for biologists as it can be easily cultivated and hence the first discovery of hydrogen metabolism in the early 1950th by Hans Gaffron was achieved with it

[32].

The lipid content of *Scenedesmus obliquus* is estimated at 40–55% DW of body mass (Table 1). Especially, the presence of palmitate and oleate as the main constituents the lipid in *S. obliquus* makes it an appropriate feedstock for biodiesel production [33]. Like many other microalgal species, this species has the ability to manipulate its metabolism in response to simple manipulations of the chemical composition of its culture medium [34] with consequent high lipid productivity. Mandal and Mallick [33] reported lipid accumulation in *Scenedesmus obliquus* could be improved under certain culture conditions such as optimizing nitrate, phosphate, and thiosulphate. Hence, under N-deficient culture the lipid content enhanced to 43% of its dry weight, in contrast to only 12.7% in the control [33]. The likely explanation could be that under nitrogen limitations the existing nitrogen is utilized for the production of enzymes and vital cell structures. Moreover, while the rate of production of all cell components is lower under nutrient limitation, oil production seems to remain higher, leading to an accumulation of oil in the cells [17]. Therefore, any CO_2 subsequently fixed is converted into carbohydrate or lipid rather than protein. For instance, Sheehan et al. [17] reported that under P-deficiency and thiosulphate supplementation, the lipid content increased up to 30% of the dry weight. Exposing algal cells to trace amount of ethyl-2-methyl acetoacetate offers a feasible method to enhance triacylglycerol production in *Scenedesmus* sp [35]. Moreover, the growth and lipid accumulation properties of *Scenedesmus* species also show variation under different cultivation temperature [see 36].

This species was highly engaged in coupling biofuel production with CO_2 mitigation strategies and wastewater treatment (see Section 3). So far, many achievements were documented in genetic and metabolic engineering using this species and also manipulating culture conditions enabled enhanced biomass production. Ren et al. [37] found the transgenic strain (R-16) can grow well heterotrophically without light, accumulating total lipid content and biomass up to 43.4% and 3.46 g L^{-1} , respectively. On top of this, under nitrogen deficient condition, the lipid accumulation is enhanced to 52.6% dry weight in this mutant strain. Jaeger et al. [38] generated the first starchless mutants of *Scenedesmus obliquus* using UV mutagenesis that show enhanced triacylglycerol content (49.4% of dry weight of the body) under photoautotrophic, nitrogen deficient conditions which paved the way towards a more feasible microalgae-driven triacylglycerol production platform. Breuer et al. [39] produced the most promising mutant of *Scenedesmus obliquus* in which the triacylglycerol content increased to $57 \pm 0.2\%$ of dry weight, in controlled photobioreactors.

Scenedesmus is also known to either take up or produce hydrogen gas depending on the partial hydrogen pressure. Hydrogen production occurs under anaerobic conditions both in the dark and in the light. Hydrogen production in the dark is supposed to be connected to fermentation. Wunschiers [31] demonstrated the co-production of hydrogen and oxygen in anaerobic condition graphically as measured by gas chromatography (Fig. 1). In the light, hydrogen production ceases directly and oxygen is produced by photosynthesis. In order to

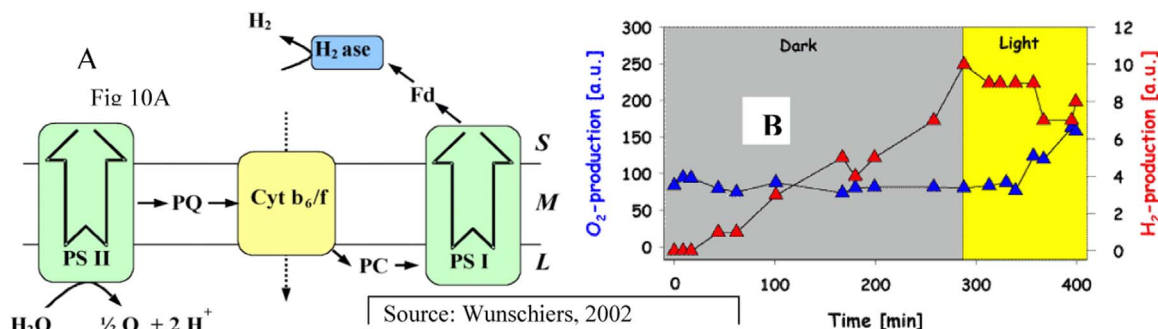


Fig. 1. Representation of hydrogenase connection to the PSs (A) and H_2 production (B) (Used with permission from wunschiers@hs-mittweida.de).

Table 2
Algae Cultivation systems.

Open ponds (raceways)	Photobioreactors (PBR)
- Accounts for > 90% of current worldwide production - less expensive to build and operate, more durable - Subject to a difficult control of temperature (day/night, seasonal) and evaporative water loss - Subject to contamination from predator strains - Require larger amount of nutrients (N, P, K) and use more energy to homogenize nutrients and the water level cannot be kept much lower than 15 cm (or 150 L/m²) for the microalgae to receive enough solar energy to grow.	- excellent for high-value products and lead to more concentrated solutions > 100 times too expensive for biofuels - offer better control over culture conditions and growth parameters (pH, temperature, mixing, CO₂ and O₂), prevent evaporation - Offer a safer and protected environment, preventing contamination or minimizing invasion - Allow easier and accurate provision of nutrients (N, P) and need larger amounts of energy for mixing and to maintain temperature - flexible systems that can be optimized according to the biological and physiological characteristics of the algal species being cultivated, allowing one to cultivate algal species that cannot be grown in open ponds.

produce hydrogen in the bioreactor, the algae need to be anaerobically adapted. Adaptation to anaerobic condition can be done in two different ways: either by flushing the algae with nitrogen gas for 3 h or placing them in the dark over night. In the former case the oxygen is simply replaced by nitrogen, while in the latter the oxygen is used by the algae through respiration. After anaerobic adaptation, addition of some grams of sodium-dithionite to the culture is required so that it will remove the remaining oxygen and the oxygen produced by photosynthesis during the experiments. Although not yet clear, after anaerobic adaptation, it is postulated that changes in the redox environment activate the inactive hydrogenase, i.e. the thioredoxin affects the activity of the hydrogenase.

2.2. Production of microalgal biomass

Microalgal biomass production is usually more costly than growing crops. The cost can be minimized if microalgal biomass productions rely on freely available sunlight although there are daily and seasonal variations in light levels. Moreover, coupling with other uses, targeting high value products and using residual byproducts can boost production economics [40]. From this point of view, tropical countries like Ethiopia with abundant sunshine could be an ideal place for the cost-effective production of microalgal biomass. In fact, Rodolfi et al. [41] reported that *Nannochloropsis* sp. has the potential for the production of more than 30 t of lipid per hectare per year in sunny tropical areas whereas only 20 t can be produced under the Mediterranean climate.

Microalgal biofuels are promising and environmentally friendly alternative renewable energy sources as they are free of the major drawbacks associated with oil crops. But the commercial level production of microalgae biofuels has still not been feasible due to the low biomass concentration. The viability of their production can be achieved by designing advanced photobioreactors, developing low cost technologies for high biomass harvesting, drying, and oil extraction. Commercial-scale production can also be realized by improving the genetic engineering strategies to adapt environmental conditions and by engineering metabolic pathways for high lipid production [4].

Thus, for commercial scale production of microalgal biofuel, we need to use our ingenuity and take advantage of existing resources such as agricultural runoff, wastewater effluent, emissions from coal-burning power plants, and even leftover material from the algal biodiesel production facilities [42]. A geothermal-powered and -heated plant built for biodiesel production from algae oil is another fascinating achievement that represents a cost-effective approach [43] Li et al. [44] reported the feasibility of expanding heterotrophic *Chlorella* fermentation for biofuel production at the industry level in a bioreactor, and suggested that high cell density cultivation strategy in bioreactors might help further cut down the cost. These authors found out that the lipid content was sharply increased by metabolic engineering in the course of heterotrophic growth of *C. protothecoides*. The recently proposed novel approach that involves the use of engineered respiro-fermentative metabolism for the production of biofuels and biochem-

icals from fatty acid-rich feedstocks [45] may be used for further improvements of the economics of microalgal biofuel production. The proposed respiro-fermentative metabolism enables the efficient synthesis of fuels and chemicals from fatty acids. Moreover, the techniques for the culture and enhancement of oil-rich, promising strains of microalgae has been of particular interest in the cost-effective development of feedstock [41]. With these packages, microalgal biofuel production will ultimately be the most efficient biofuel production, both in terms of land use and energy conversion [3].

2.2.1. Cultivation systems

Cultivation of microalgae seems quite easy as only simple nutrients need to be provided. Most research on microalgae cultivation has focused on growing algae in clean but expensive photobioreactors, or in open ponds, which are cheap to maintain but prone to contamination [6]. Therefore, currently two main microalgal cultivation systems are adopted: open ponds and photobioreactors [46]. Their advantages and disadvantages have been listed in Table 2. Photobioreactors could be constructed in different shapes: flat-plate, tubular or pyramidal photobioreactor. The *Pyramid* photobioreactor has advantage over the other design in that it is using fully controlled and automatic system that increases the production rate and it enables to grow any microalgae at any climate conditions [47].

2.2.2. Enhancing algal biology to reduce production costs

Although biofuels made from microalgae hold the potential to solve many of the sustainability challenges facing other biofuels today, the production of their biomass using the technology available is not economical and, the existing algal species could not be grown sufficiently cheaply and, at the same time, produce oil usable as a source of fatty acids for biodiesel [48]. Based on conventional estimates, algal biofuels produced in large volumes with current technology would cost more than \$8 per gallon (in contrast to \$4 per gallon for soybean oil today) [17]. Therefore, producing low-cost microalgal biodiesel requires either improvements of algal biology through genetic and metabolic engineering or modifying the culture conditions in some species or combination of both [3]. Concomitant use of tailor-made, rather than wild type, algal strains may help to reduce production costs to a level that could bring algal oil within the reach of economic feasibility [48].

To address these drawbacks, a number of research works have been made for decades. The last few years have witnessed significant progress in the genetic engineering of microalgae. Transgenesis in algae is a fast-growing technology as selectable marker genes, promoters, reporter genes, transformation techniques, and other genetic tools and methods are already available for various species [50]. Outstandingly, the commercial application of algal transgenics is beginning to be realized, and algal biotechnology companies are being established.

Microbial production of natural products has been achieved by transferring product-specific enzymes or entire metabolic pathways

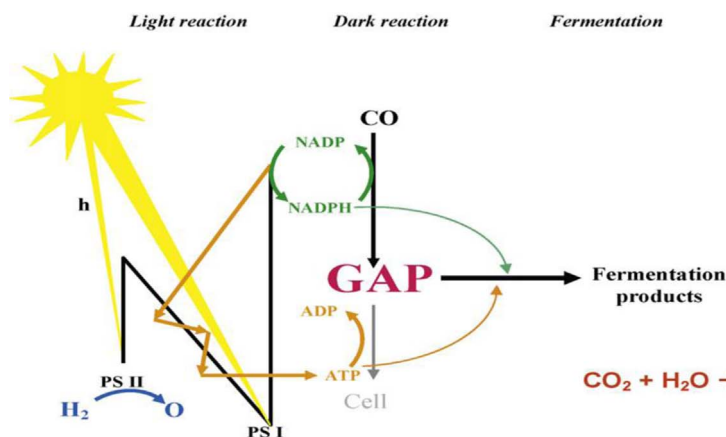


Fig. 2. Schematic representation of Photanol process [49]. (Cited after communication by e-mail with Hellingwerf, K.J.Hellingwerf@uva.nl).

from rare or genetically intractable organisms to those that can be readily engineered [51]. Production of special chemicals, bulk chemicals, and fuels has been made possible by combining enzymes or pathways from different hosts into a single microorganism and by engineering enzymes to have new function [51]. On the other hand, Hellingwerf and de Mattos [49] have proposed the photanol approach: Light-driven conversion of CO_2 and water into biofuel can be achieved by combining the light reactions of photosynthesis and the Calvin Cycle with a fermentative pathway from a chemoheterotrophic microorganism in one single chimera (an organism with at least two genetically different components resulting from mutation, the grafting of plants, or the insertion of foreign cells into an embryo) (see Fig. 2). This would circumvent the need for converting CO_2 into the complex mixture of biopolymers (protein, nucleic acids, cell walls, neutral and phospholipids, etc.) and then applying a series of subsequent processing steps to convert this complex mixture into a specific biofuel with consequent increase in the overall efficiency of the biofuel production process.

Genetic and metabolic engineering and transgenics offer the potential to increase the lipid productivity of microalgae and contribute to improving the economics of production of microalgal diesel [24]. In this regard, many improvements have been realized, including increased lipid and carbohydrate production, improved H_2 yields, and diversion of central metabolic intermediates into biofuels [52]. Some of the achievements in genetic and metabolic engineering that involve *Scenedemus obliquus* have been discussed/presented in previous Section 2.1.1, while some more with other microalgal species are presented here. The transgenic *Chlamydomonas reinhardtii* is now being developed for various biotechnological applications, including the production of biohydrogen [53]. In this species, a mutant created by the genetic blockage of starch synthesis showed increased accumulation of lipids on a cellular basis during nitrogen deprivation. In an attempt to increase algal productivity through down-regulating expression of the light-harvesting antenna complexes in *C. reinhardtii*, the transgenic alga showed higher resistance to photo-oxidative damage with a concordant 30% increase in photosynthetic efficiency [14]. Another fascinating finding in this scenario is an engineered blue green alga, *Synechococcus leopoliensis*, equipped with the cloned bacterial cellulose synthase genes from *Gluconobacter xylinus*. This engineered alga produces extra-cellular deposits of non-crystalline cellulose, a polymer which is ideal as a feedstock for biofuel production of various alcohols [49].

Outstanding achievements were reported in enhancing alcohol production via genetic engineering in two independent cyanobacterial strains (*Synechococcus* sp.) through expression of pyruvate decarboxylase and alcohol dehydrogenase II genes [54]. This was done through cloning the code sequences of pyruvate decarboxylase (*pdc*) and alcohol dehydrogenase II (*adh*) from the bacterium *Zymomonas mobilis* into the shuttle vector (referred as pCB4) and then used to transform the

cyanobacterium *Synechococcus* sp. (strain PCC 7942). The cloned and transformed cyanobacterium synthesized ethanol, which diffused from the cells into the culture medium. Another important recent finding was that of Joule Unlimited (see www.jouleunlimited.com), where the engineered/transformed strain was able to secrete ethanol at a rate of $1 \text{ mg l}^{-1} \text{ h}^{-1}$, which greatly outpaces rates reported previously ($0.2 \text{ mg l}^{-1} \text{ day}^{-1}$). Still other important finding reported by Atsumi et al. [55] which involves isobutanol production, as isobutanol possesses greater energy density. In this work the valine synthesizing enzymes have been used to divert internal pyruvate stores to the precursor 2-ketoisovalerate, which is converted to isobutyraldehyde- a precursor of isobutanol-at a rate of $6.23 \text{ mg l}^{-1} \text{ h}^{-1}$ by co-expression of ketoacid decarboxylase (*kivD*) within *Synechococcus elongates*. Furthermore, Schirmer et al. [56] identified two enzyme families that are responsible for alkane production in cyanobacteria: an acyl-acyl carrier protein reductase and an aldehyde decarboxylase. This discovery opens up unexplored possibilities for optimization of biodiesel production in cyanobacteria. In this connection, Ducat et al. [57] argued that genetic approaches to construct blue green algal strains with a higher and special photosynthetic efficiency could be used to improve product yields from several biochemical pathways.

In the arena of improving the photosynthetic productivity and light utilization efficiency of the algae, some achievements are also recorded, as these are important factors in determining production cost [58]. At high photon flux densities, the rate of photon absorption by the chlorophyll antenna far exceeds the rate at which photons can be utilized for photosynthesis. Thus, microalgal mass cultures growing under full sunlight have a low per chlorophyll productivity resulting in excess photons (up to 80%) dissipated as fluorescence or heat. Consequently, this reduces light conversion efficiencies and cellular productivity to fairly low levels. Polle et al. [58] suggested that this shortcoming could possibly be alleviated by the development of microalgal strains with a limited number of chlorophyll molecules in the light-harvesting antenna of their photosystems, i.e. strains that have a truncated chlorophyll antenna size. It is documented (see [59]) that the truncated chlorophyll antenna size minimizes absorption and wasteful dissipation of sunlight by individual cells, resulting in better light utilization efficiency and greater photosynthetic productivity by the green alga mass culture

In general, metabolic and molecular level engineering can be potentially useful to promote several desirable features of microalgae [14]: to increase photosynthetic efficiency to enable increased biomass yield; to enhance biomass growth rate; to increase oil content of biomass; to improve temperature tolerance to reduce the expense of cooling; to eliminate the light saturation phenomenon so that growth continues to increase in response to increasing light level; to reduce photoinhibition that actually reduces growth rate at midday light intensities that occur in temperate and tropical zones; and to reduce

susceptibility to photooxidation that damages cells.

2.3. Types of microalgal biofuels and their production

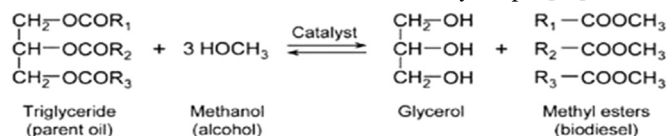
Microalgae can supply several different types of renewable biofuels such as biodiesel derived from microalgal oil [13,17]; biohydrogen produced photobiologically [31,53]; biomethane produced by anaerobic digestion of algal biomass [60]; bioethanol produced via fermentation and anaerobic digestion of the remaining algal biomass [61]. The energy production from microalgal biomass involves biochemical, thermochemical, chemical and direct combustion processes.

The whole process involves several steps. The story begins with the production of microalgal biomass by using light, carbon dioxide, water and inorganic nutrients. The next is harvesting, where the cells are separated from the water and residual nutrients. In the third step, the algal oil is extracted from the recovered biomass. In the fourth step, most of the biomass undergoes anaerobic digestion, which produces biogas to generate electricity. Most of the power generated from the biogas is consumed within the biomass-production process and any surplus energy can be sold to grid. In this process, carbon dioxide emissions from the power generation stage are fed into the biomass production. Thus, the whole process remaining carbon neutral, as all the power needed for producing and processing the algae would come from biodiesel itself and from methane produced by anaerobic digestion of biomass residue left behind after the oils has been extracted.

Oil productivity of many microalgae greatly exceeds the oil productivity of the best oil producing crops (Table 3). Moreover, while the lipid content of microalgae, on a dry weight basis, varies between 20% and 40%, lipid contents as high as 85% have been reported for certain microalgal strains [3,62]. Satyanarayana et al. [12] and Singla et al. [63] postulated that in contrast to oil-producing crops, microalgae have more potential to completely displace petroleum-derived transport fuels without adversely affecting supply of food and other crop products, as their production can be situated on non-arable land [12,63].

2.3.1. Microalgal biodiesel

One of the most efficient ways to produce microalgal biofuel is through trans-esterification of the algal oils to produce biodiesel [17]. The biodiesel trans-esterification reaction is very simple [54]:



Biodiesel production involves mixing triglycerides, methanol, and catalyst (may be alkali such as potassium hydroxide or acid) in a

Table 3
Yield per acre of various feedstocks.

Source	Oil Yield (l/ha)	Area to produce global oil demand (ha × 10 ⁶)
Corn	172	1540
Cotton	325	15,002
Soyabean	446	10,932
Mustard seed	572	8524
Sunflower	952	5121
Canola	1190	223
Rapeseed/Canola	1190	4097
Oil Palm	1892	2577
Jatropha	5950	819
Algae (10 g/m ² /day at 30% TAG)	12,000	406
Algae (50 g/m ² /day at 50% TAG)	98,500	49

controlled reaction chamber to promote trans-esterification. The initial product is placed in a separator to remove the glycerine by-product; the excess methanol is recovered from the methyl esters through evaporation; and the final biodiesel is rinsed with water, pH neutralized, and dried [20]. Unlike petroleum fuels, the relative simplicity of biodiesel fabrication makes its production scalable.

The residual biomass from biodiesel production processes, lipid-extracted microalgal biomass residues (LMBRs), is rich in carbohydrates and proteins. Thus, LMBRs are possible substrates for dark fermentation to produce hydrogen. Yang et al. [65] reported that it was difficult to directly convert LMBRs to hydrogen, probably because the complex oil extraction processes (dewatering, drying, destruction of the cell wall and extraction with solvents) caused them to resist biodegradation. They also argued that the thermo-alkaline pretreatment improved the LMBR solubilization leading to an increase in hydrogen production. This form of converting LMBRs into hydrogen serves the dual role in renewable energy production and sustainable development of microalgal biodiesel industry.

Alternatively, the production of methane via the anaerobic digestion of microalgal biomass residues of the biodiesel production process has the potential to meet some of the energy requirements of the process of converting primary biomass to fuel. Otherwise, the residual biomass would be considered as waste with its disposal cost increasing the economics for biodiesel production from microalgae [3]. Therefore, the extraction of energy from the residual biomass could serve as a way to maximize energy production from microalgae, and reduce the overall process costs and wastes [66]. Dimitrov [67] also suggested that the non-lipid portion of the algal biomass may be an option for electricity generation. Furthermore, glycerol, the byproduct of biodiesel production through trans-esterification, can be used to produce hydrogen via anaerobic fermentation. Biodiesel can be used as either direct substitute or as an extender to fossil diesel fuel in compression ignition engines and has the potential to reduce carbon dioxide, hydrocarbon and carbon monoxide emissions. The quality of the fuel product is equivalent to petroleum diesel and can be incorporated into the existing fuel infrastructure with negligible change [68].

2.3.2. Biohydrogen from microalgae

Hydrogen gas is considered as a potential future energy carrier by virtue of the fact that it is renewable, does not evolve CO₂ in combustion, liberates large amounts of energy per unit weight in combustion, and is easily converted to electricity by fuel cells. It is currently produced by fossil fuel-based processes, which emit large amounts of CO₂, and relatively smaller amounts of other air pollutants such as sulfur dioxide and nitrogen oxides. Miyamoto [69] noted that biological H₂ production has several advantages over hydrogen production by photoelectrochemical or thermochemical processes, and thus recently received renewed attention owing to air pollution and global warming concerns. Photosynthesis involves biological electrolysis; breakdown of water into hydrogen and oxygen. In normal photosynthetic pathway, the hydrogen is subsequently combined with carbon dioxide to form carbohydrate. But, in some algae and under certain special conditions, hydrogen is released instead of carbohydrate during photosynthesis [31,70,71].

Several unicellular green algae have the capacity to produce H₂ by using water and sunlight as an energy source (Fig. 3). Over 70-years ago, Gaffron and Rubin [32] discovered that green algae such as *S. obliquus* and *Chlamydomonas reinhardtii* could, depending on experimental conditions, either assimilate or photo-produce molecular hydrogen (H₂). They observed that the algae would sometimes switch from the production of oxygen to the production of hydrogen. But, they did not explain the mechanism for this change. In the late 1990s, Melis [53] found that depleting the amount of sulfur available to the algae interrupted its internal oxygen flow, allowing the hydrogenase to produce hydrogen. Chochois et al. [72] described direct photolysis mechanism for hydrogen production in *C. reinhardtii* and discussed

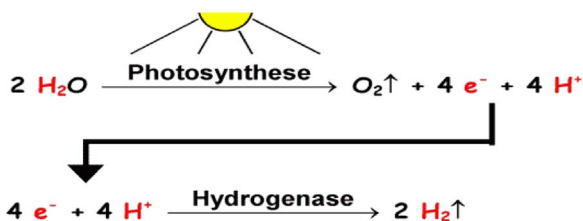


Fig. 3. The reactions involved in photohydrogen production [31]. (Used with permission from wuenschi@hs-mittweida.de) http://www.uni-koeln.de/~aei53/PhotoHydrogen/biore_03.htm.

the possibility of sustainable production. When *C. reinhardtii* cells are illuminated after adaptation to anaerobic conditions, electrons originating from water splitting at PSII are driven by the photosynthetic electron transport chain to ferredoxin and to a reversible iron hydrogenase, thereby enabling the production of H_2 from water and solar energy (Fig. 3).

As hydrogenase is sensitive to O_2 [73] and because O_2 is produced at PSII, hydrogen photoproduction stops after a few minutes of illumination. To alleviate this problem and allow long-term hydrogen production, an experimental protocol based on sulfur deprivation was proposed [53]. The consequent anaerobiosis enables to reduce the oxygenic PSII activity, and to remove the generated oxygen by keeping the cell's respiration rate on exogenous acetate and/or endogenous carbohydrates [74]. Once the photosynthetic oxygen generation rate is lower than the rate of O_2 -uptake by respiration, the cells culture gradually becomes anaerobic thereby inducing the activity of reversible hydrogenase [75]. This protocol depends on a two-phase process: the first phase involves oxygenic photosynthesis that drives production of biomass and carbohydrate stores, and the second phase involves anaerobic stage, where the hydrogenase is induced and hydrogen is produced. Using this two-phase process and same species (e.g. *C. reinhardtii*), Pantí et al. [76] demonstrated the possibility of producing hydrogen in a low-cost system by solar radiation in a closed photobioreactor. These authors pointed that in external conditions with solar radiation and without temperature control, this technique represents the potential for larger scales production.

The latest and more powerful technique for immobilizing H_2 photoproducing green algae was developed using a thin alginate film [75]. Alginate films with entrapped sulfur/phosphorus-deprived *C. reinhardtii* exhibited higher cell density, higher specific rates (up to $12.5 \text{ mmol mg}^{-1} \text{ Chl h}^{-1}$) of H_2 evolution, light conversion efficiencies to H_2 of over 1% and high resistance of the H_2 photoproducing system to inactivation by atmospheric O_2 . Kosourov and Seibert [75] reported that the reduced susceptibility of the immobilized microalgal H_2 -producing system to inactivation by O_2 depends on the presence of acetate in the medium, which maintains higher rates of respiration and the capacity of the alginate polymer itself to effectively separate the entrapped cells from O_2 in the culture.

Many researchers come up with genetically modified strains which showed improved hydrogen production. Faraloni and Torzillo [77] reported mutants of *C. reinhardtii* possessed salient features over the wild type, for hydrogen production: i.e. a reduced amount of chlorophyll both per dry weight and per cell; a higher respiration-to-photosynthesis ratio, which reduced the length of the aerobic phase; a greater capacity to synthesize carbohydrates; and a longer period of hydrogen production.

Following Gaffron and Rubin's discovery [32], basic researches on the mechanisms involved in hydrogen production have determined that the reducing power (electron donation) of hydrogenase and nitrogenase do not always come from water, but may sometimes originate from the intracellular energy reserve including carbohydrates [78]. The stored energy is released through fermentation of the endogenous carbohydrates in dark conditions, and the excess reducing power can be deposited by hydrogenase on protons (H^+) forming molecular

hydrogen. Fouchard et al. [79] have reported clear correlation between the starch content decrease and the kinetics of H_2 production, indicating a central role of starch in H_2 production. In this second mechanism, the indirect biophotolysis consists of two stages in series: photosynthesis for carbohydrate accumulation, and dark fermentation of the carbon reserve for hydrogen production. In this way, the oxygen and hydrogen evolutions are temporally and/or spatially separated. This separation not only avoids the incompatibility of oxygen and hydrogen evolution, but also makes hydrogen purification relatively easy because CO_2 can be conveniently removed from the H_2/CO_2 mixture.

The ability of microalgae to photosynthetically generate molecular H_2 has enthralled the fascination and interest of the scientific community because of the fundamental and practical importance of the process [80]. In green algae hydrogen gas evolution was observed only after prior adaptation to an anaerobic condition, whereas in some blue-green algae the formation of hydrogen gas was observed in both an anaerobic environment and in air [81]. In the heterocystous blue-green alga, *Anabaena cylindrica*, hydrogen has been formed without prior adaptation but only in an anaerobic environment and in the absence of dinitrogen. This alga is capable of dinitrogen fixation in air, because heterocyst cells protect the oxygen-labile nitrogenase enzyme against oxygen. Therefore, these algae appear to offer potential for work aimed at manipulating cultures to allow hydrogen production in air. In non-nitrogen fixing cyanobacteria, hydrogen evolution is also observed when the cells are exposed to a reducing atmosphere consisting of CO and C_2H_2 under a low light intensity ($4\text{--}7 \text{ W/m}^2$) [82]. In the absence of nitrogenase, a bi-directional hydrogenase is most likely responsible for hydrogen evolution in those strains [83].

Developing a realistic biophotolysis system requires a plan of a low-cost culture and H_2 collector system, as well as selection of the most suitable strain of algae. One of the aforementioned requirements of practical biosolar converter alga may be its resistance to high temperatures. For these reasons, Miyamoto et al. [69] suggested the thermophilic blue-green alga *Mastigocladus laminosus* over *Anabaena cylindrica*, for H_2 production. Amos [84] performed cost analysis of photobiological hydrogen production from *C. reinhardtii* assuming a continuous hydrogen production system within a reactor. Accordingly, he found out photobiological hydrogen production from *C. reinhardtii* would be cost-effective given that the system is directly connected to a hydrogen pipeline and the production is not limited by storage capacity, and the cost of photo-bioreactor is maintained low. Later, Sen et al. [85] noted the net analysis of energy showed directions for viability and the possibility of increasing the production rates and yields by using techniques like metabolic flux analysis and genetic engineering. In this regard, the advances in the field of algal hydrogen production follow two fundamental approaches: i) the temporal separation of the reactions of O_2 and H_2 production in green algae, and ii) targeting on genetic engineering to increase the O_2 tolerance of the reversible hydrogenase enzyme [74].

2.3.3. Bioethanol from microalgae

Bioethanol, produced from renewable biomass such as sugar and starch materials, is believed to be one of the alternative energy sources, and is currently being harnessed extensively. Nevertheless, the utilization of sugar and starch materials as feedstocks for bioethanol production creates a major competition with the food market in terms of land for cultivation, and this makes bioethanol from these sources economically less attractive.

Although the majority of microalgae have relative low carbohydrates content, their biomass composition can be manipulated by applying various stress cultivation conditions (nutrient starvation or high light intensity), and some have enhanced carbohydrates accumulation up to a content of up to 65% of their biomass [86,87]. Following this, many microalgal species have been endorsed for bioethanol production either for their suitable body composition (i.e. high starch

content) or through genetic modification. *Chlorella vulgaris* is a good source of ethanol due to the high starch content (ca. 37% dry weight), for which up to 65% ethanol conversion efficiency has been reported [88]. While Ho et al. [89] demonstrated increased accumulation of carbohydrates when *Chlorella vulgaris* was cultivated under nitrogen deficient conditions, Miranda et al. [90] did the same with *Scenedesmus obliquus*. Choi et al. [91] and Nguyen et al. [92] cultivated the green alga *Chlamydomonas reinhardtii* under high illumination of 450 $\mu\text{E}/\text{m}^2\text{s}$ and found a carbohydrate content of about 60% of dry biomass. Harun et al. [61] reported that *Chlorococcum* sp. is suitable as a substrate for bioethanol production via yeast (*Saccharomyces bayanus*) under different fermentation conditions, with a productivity level of 3.83 g L⁻¹. Markou et al. [87] reported optimum acid concentration (0.5 N) for carbohydrate saccharification and yielded about 16% g ethanol per g of dry *Arthrospira platensis* fermented by *Saccharomyces cerevisiae*. Deng and Coleman [54] reported the mutants of *Synechococcus* sp. synthesized ethanol (see Section 2.2.2). These authors suggested cyanobacteria as right-choice for bioconversion of solar energy and CO₂ into a valuable resource (ethanol) because of their simple growth requirements and efficiency. It seems that this has attracted more recent attention because in an attempt to develop economically competitive technology for the direct production of bioethanol from a metabolically engineered strain of *Synechocystis* sp. PCC 6803 in photobioreactors, European Union financed a project entitled Direct Ethanol from Microalgae (DEMA) (<http://www.dema-etoh.eu/en/>).

2.3.4. Biomethanol from microalgae

The application of algae in the search for sustainable renewable energy in the form of biomethanol was proposed long ago. Golueke and Oswald [93] argued that hypothetical systems designed to overcome certain disadvantages of solar energy systems involved algae. According to Golueke and Oswald [93], the hypothesized systems involve the transformation of solar energy into the cellular energy of algae; the cellular energy of the algae would in turn be converted to the chemical energy of methane through anaerobic fermentation of algae by bacteria. As a fuel, bio-methanol can either be blended with petrol, or used as a feedstock for other environmentally friendly fuels.

Compared to other hydrocarbon fuels, burning methane produces less carbon dioxide for each unit of heat released. It is reported that, at about 891 kJ/mol, methane's combustion heat is lower than any other hydrocarbon; but the ratio of the molecular mass (16.0 g/mol) to the heat of combustion (891 kJ/mol) shows that methane, being the simplest hydrocarbon, produces more heat per unit mass than other complex hydrocarbons. Fermentation of methane offers an effective means of pollution reduction, superior to that achieved via conventional aerobic processes.

2.4. Harvesting methods of microalgae

Once the desired biomass is produced, the next challenges is harvesting of algal biomass which is estimated to be over 30% of the total cost in case of algal production in open ponds [Zittelli et al. 2006 cited in 94]. Microalgae harvesting is a two stage process involving bulk harvesting (separation of biomass from the bulk suspension) and thickening (concentrating the slurry) [95]. This step is a serious bottleneck in current biofuel technology owing to the small size of microalgae and low density, ending up in escalating the capital cost. Therefore, the selection of methods for dewatering and harvesting of algal biomass with a minimum cost is demanding. Currently harvesting and recovery of microalgal biomass are carried out with one or more of the following techniques: flocculation, centrifugation, filtration, and flotation.

2.4.1. Flocculation

Flocculation involves a process of forming aggregates that are often

performed as a pretreatment to increase the cell density by natural, chemical, or physical means [96]. Chemical flocculants either inorganic flocculants such as Zn²⁺, Al³⁺, Fe³⁺ [97,98] or organic flocculants such as chitosan [99] or starch [100] are usually added to induce flocculation. The surface charge of microalgal cells is generally negatively charged due to the ionization of functional groups on the microalgal cell walls and also by the adsorption of ions from the culture medium which are neutralized by applying positively charged electrodes or cationic polymers, also help in flocculating the microalgal biomass. Algal flocculation with cationic polymers is most effective at low pH levels but influenced by the growth phase of the algae, with optimal conditions for flocculation in the late log and early declining growth phases. This harvesting method is expensive because of the cost of flocculants; hence, if flocculation method is used, flocculants need to be inexpensive, easily produced, and nontoxic.

Microalgal biomass harvesting can also be carried out by flocculation induced through changing the culture conditions such as extreme pH, nutrient depletion, temperature changes and changes of the level of dissolved O₂. Wu et al. [101] reported that raising the medium pH induced 90% efficiency of flocculation in three microalgae species (*Chlorella vulgaris*, *Scenedesmus* sp., *Chlorococcum* sp.). The mechanism was explained as Mg²⁺ in the growth medium hydrolyzed to form magnesium hydroxide precipitate, which coagulated microalgal cells by speeding-up flocculation and charge neutralization. On the other hand, Liu et al. [102] proposed a new flocculation method by decreasing pH value (rather than increasing) of growth medium, which resulted in the flocculation efficiencies of up to 90% for *Chlorococcum nivale*, *Chlorococcum ellipsoideum* and *Scenedesmus* sp. The accompanying explanation for the flocculation mechanism was that at lower pH the carboxylate ions of organic matters adhere on microalgal cells accepting protons and neutralizing negative charges, thereby resulting in disruption of the dispersing stability of cells and subsequent flocculation of cells. However, many authors complain that this method may induce undesired changes in cell composition and thus should not be preferred.

Another option for further cutting-down the cost of harvesting microalgal biomass is application of bio-flocculation method. This method can be a cost-effective and energy-efficient alternative and it involves the application of one flocculating microalga to concentrate the non-flocculating microalga of interest [94]. Salim et al. [94] reported that when co-cultured, the flocculating microalgae (*Scenedesmus obliquus*) improved the sedimentation rate of the accompanying microalga (*Chlorella vulgaris*) and increased the recovery of biomass. In bio-flocculation method, there is no addition of chemical flocculants and also similar cultivation conditions can be used for the flocculating microalgae as for the microalgae of interest that accumulate lipids. The method is as easy and effective as chemical flocculation, yet in contrast it is sustainable and cost-effective as no costs are involved for pre-treatment of the biomass for oil extraction and for pre-treatment of the medium before it can be re-used.

2.4.2. Centrifugation

Centrifugation is a process of separation based on the size of particle and density. Its separation efficiency depends upon the size of desired algal species. According to Shelef et al. [103] different centrifugal techniques have been employed in various types and sizes depending on the uses such as tubular centrifuge, multichamber centrifuges, imperforate basket centrifuge, decanter, solid retaining disc centrifuge, nozzle type centrifuge, and solid ejecting type disc centrifuge. Although centrifugation method is very effective and rapid, it is considered unfeasible in large-scale algal culture system due to the high capital and operational costs [104] and an energy-intensive process [105].

2.4.3. Filtration

Microalgal biomass can also be harvested by filtration through

filters on which the algae accumulate and the liquid medium passes through. The forms of filtration vary based on techniques of filtration used such as pressure filtration, vacuum filtration, tangential flow filtration etc [106]. Depending on the pore size of filters the filtration systems can be designated as macrofiltration (pore size of $> 10 \mu\text{m}$), microfiltration (pore size of $0.1\text{--}10 \mu\text{m}$), ultrafiltration (pore size of $0.02\text{--}2 \mu\text{m}$), and reverse osmosis (pore size of $< 0.001 \mu\text{m}$) [107]. This method of collecting microalgal biomass is very time consuming and also requires high running costs.

2.4.4. Flotation

The microair bubbles created on the surface may trap microalgal cells and thus the cells float at the surface of water [108]. The efficiency of biomass harvesting by flotation method is dependent on the size of the bubbles created which may be nanobubbles, microbubbles, and fine bubbles [109]. Although flotation has been mentioned as a potential harvesting method which does not require any addition of chemicals, there is very limited evidence of its technical or economic viability [110].

2.5. Microalgal oil extraction

Oil extraction is another bottleneck in algae-to-biofuel industries as it is also incurring considerable cost and effort. Microalgal oil extraction is carried out by chemical methods, physical methods, or a combination of the two. It is estimated that although the cost to extract oil from microalgae may vary, it is likely to be around three times higher than that of extracting palm oil [3].

2.5.1. Physical extraction

Microalgal oil is separated from non-oil biomass through mechanical crushing. The algal material is dried and then pressed with an oil press so that the oil content is separated. Oil pressing methods vary depending on strains of algae under consideration such as using screw, expeller and piston. In the case of many commercial manufacturing of vegetable oils, a combination of mechanical pressing and chemical solvents are used in extracting oil, which is also adopted for microalgal oil extraction. For larger commercial scale extraction, specially designed disruption vessels, with a continuous flowing stream of material to be disrupted are used [111].

2.5.2. Chemical extraction

The chemical extraction of oil which usually follows mechanical disruption of algae cells can be performed with solvents. A common solvent used in oil extraction process is hexane, which is widely used in the food industry and is relatively inexpensive. Benzene and ether can also be used to separate oil. At the laboratory scale, where often no mechanical disruption precedes, this can be done by the method of Bligh and Dyer [112]. This process uses a polar and a non-polar solvent to extract the corresponding lipid fractions from the cells. The disadvantages of using solvent extraction on a commercial scale are that it requires extra energy input and it has the potential to contaminate the algae solids, thereby restricting options for their end use. Moreover, care must be taken to avoid exposure to vapors and skin contact, either of which can cause serious health damage.

2.6. Trans-esterification of algal oils

Owing to their high viscosity, oils (both vegetable and microalgal) can not be directly applied to engines [Fuls et al., 1984 cited in 113] and hence the trans-esterification is intended to reduce the viscosity and increase the fluidity. Trans-esterification is carried out in three different ways: base catalyzed transesterification, acid catalyzed transesterification and noncatalytic trans-esterification.

3. Other applications of microalgae coupled with biofuel production

Since the first use of microalgae (such as *Nostoc*) for survival during famine in China about 2000 years back [60] and the first notion of application of microalgae in the production of biogas in the fifties [110], and the later proposal as a source of different types of fuel, several application areas have been identified [12]. Combination of various applications like coupling the microalgal biomass production for biofuel with wastewater treatment and sequestering flue gases from industries will serve many purposes and can be more economical. A number of works have already been documented for the aforementioned activities. Yun et al. [114] reported *Chlorella vulgaris* cultured in wastewater discharged from a steel-making plant with the aim of developing an economically feasible system to remove ammonia from wastewater and CO_2 from flue gas simultaneously, showed very good performance.

3.1. Microalgae in wastewater treatment

In wastewater-treatment facilities, microalgae can be used to reduce the amount of toxic chemicals needed to clean and purify water [19]. This attribute is associated with the fact that the actively growing microalgae tend to be highly negatively charged on their surface and hence strongly adsorb polyvalent cations [115]. This notion has been corroborated by the findings of different authors who tested different microalgal species using wastewater originated from various sources. Ramani (1974 cited in [115]) reported algae cultured in the wastewater-treatment ponds produced high biomass and efficiently removed most of the heavy metals from the system. Monteiro et al. [116] compared the efficiency of metal removal in a wild strain of *S. obliquus*, isolated from a heavy metal-contaminated environment, and commercially available strain (ACOI 598) of the same microalga species. These authors obtained similar results for the maximum extent of metal removal (i.e. remove cadmium cations from aqueous solutions) for both strains and concluded that microalgae can be cultivated in waste discharges to couple waste treatment with biodiesel production. Mandal and Mallick [117] found that in *S. obliquus* the lipid pool was even boosted to 1.0 g L^{-1} against 0.1 g L^{-1} in the control. Further, the waste-grown *S. obliquus* showed an increased content of the saturated fatty acid pool, which is desirable for good-quality biodiesel. These same researchers [118] tested the suitability of *S. obliquus* in a re-circulatory aquaculture system with fish pond discharges and poultry litter to couple with waste treatment and found lipid productivity of $14,400 \text{ l ha}^{-1}\text{year}^{-1}$. Higher growth performance of the alga *S. obliquus* cultured in sweetmeat factory waste was found [119]. Ruiz-Marin et al. [120] pointed out sludge immobilized with microbes and *S. obliquus* reduced treatment cost which would otherwise incur about one-third of the total investment, with concurrent increased growth rates of algae. Furthermore, Hondafa et al. [121] reported that *S. obliquus* showed its maximum specific growth rate when cultured in olive-oil mill wastewater. More recent work [122] revealed over 90% removal efficiency of TP and TN with concomitant lipid content of 32% DW and triglycerol content increased to 148 mg L^{-1} in *Scenedesmus* sp. ZTY1 cultured in domestic wastewater. According to Oswald [115], microalgal growth and CO_2 assimilation raises the pond pH and dissolved O_2 , resulting in the removal of parasites. Therefore, wastewater fertilized algal pond offers multipurposes: biomass for biofuel; oxygen for bacterial growth; nutrient uptake; adsorption of heavy metals, thereby disinfecting pond.

3.2. Microalgae in CO_2 mitigation

The huge amounts of potentially recoverable CO_2 released from industries and other various human activities necessitate the development of technologies for sequestering or, utilizing this CO_2 . As the

concentration of CO₂ in flue gas emitted from thermal power stations or steel-making plants is about 500 times higher than that in the atmosphere, screening by microalgae which are tolerant to high concentration of CO₂ is being assessed as an essential step for the biological removal of CO₂ from the flue gas [123]. Microalgae are potential candidates for utilizing excessive amounts of CO₂, since, when cultivated, these organisms are capable of fixing CO₂ to produce energy and chemical compounds upon exposure to sunlight. Microalgae are able to fix CO₂ efficiently from different sources, including the atmosphere, industrial exhaust gases, and soluble carbonate salts [124]. Furthermore, CO₂ fixation by microalgae, coupled with biofuel production and wastewater treatment, may provide a very promising alternative to current CO₂ mitigation strategies. Thus, the photoautotrophic CO₂ fixation by algal cultures has the potential to diminish the release of CO₂ into the atmosphere, helping alleviate the trend toward global warming [125].

Various microalgal species have been documented for their high tolerance to CO₂ and efficiency of CO₂ fixation. Ono and Cuello [125] remarked that selection of optimal microalgal species to realize workable biological CO₂ fixation systems is vital. For instance, Yun et al. [114] demonstrated that after adaptation to 5% (v/v) CO₂, the growth of *C. vulgaris* was significantly improved at a typical concentration of CO₂ in flue gas of 15% (v/v). Morais et al. [126] found *Scenedesmus obliquus* and *Chlorella kessleri* grew well when the culture medium contained up to 18% CO₂. In general, the selection of optimal microalgal species depends on specific strategies employed for CO₂ sequestration. CO₂ assimilation ability is a key criterion in selecting algal species. Some other criteria include commercial values, tolerance to CO₂, high temperature and trace elements in the flue gases. The selection procedure should consider seeking additional value for the system to offset operational costs i.e. there is a need to develop multi-functional systems since economic feasibility is one of the major issues to realize biological mitigation systems. The direct utilization of power plant flue gas adds the advantage of cost-reduction of separating CO₂ gas.

4. Microalgal biofuel production opportunities in Ethiopia

Ethiopia is non-oil producing less-developed country, importing all of its petroleum products and the demand for petroleum fuel is rising rapidly. Surprisingly, it is reported that in the second quarter of 2007/08, petroleum imports exceeded the total exports earnings by 30% [127]. Ethiopia, as one of the fast growing countries, needs to look for all possible alternatives of energy to meet its escalating demands. The country has already taken steps to support the emergence of biofuel developments. In addition to a long established state ethanol project, there are now several private biofuel initiatives, both large scale plantations and outgrower schemes [128]. The current bio-fuel development in the country emphasizes on the production of bio-ethanol from sugar beet, sugar cane, sweet sorghum and others, and biodiesel

from *Jatropha*, castor, bean and palm [129]. As there are several drawbacks associated with the expansion and utilization of first and second generation biofuels, it is imperative that the country move towards third (or even fourth) generation biofuel production.

Since Ethiopia has all the aforementioned merits and potential to develop microalgal biofuel, it would be valuable to embark on this industry. Above all, the country is located in the tropics, receiving sunlight throughout the year, which is 'free' raw material for microalgal biomass production. Asmare et al. [127] has already reported that biomass and lipid productivity in the Ethiopian condition around 93 g/m²/day and 115,200 l/ha/year, respectively. Moreover, the freshwater bodies of the country are rich in microalgal species with biofuel production potential. Many of the Rift Valley Lakes harbor abundant populations of algal species with this potential. For instance, *Scenedesmus* and *Botryococcus* species are abundant in Lakes Awasa and Ziway [130] and Lake Chamo [131]. They are also found in the largest lake of Ethiopia, Lake Tana [132], and in some of the Debre Zeit Crater Lakes [133,134].

5. Promises and challenges in the algae-to-biofuel industries

Owing to their higher efficiency in converting solar energy into chemical energy (3–8%), and production of higher biomass (20–60 g/m²/day) and oil yield than conventional energy crops (only 0.5% efficiency with < 10 g/m²/day yield), microalgal biofuel production seems to be a good business to embark on [135]. Owing to these desirable features of microalgae, worldwide attention has recently been given to research activities related to microalgae and their production. The issue has attracted much consideration of many scientists and government officials in a number of countries. Scientists are devoting much of their time in microalgal research and many governments are providing a considerable sum of money for projects related to microalgae [see 136]. Though there are many challenges in microalgal biodiesel production, more and more inventors are tempted to believe that the rewards would eventually outweigh the risks and, so far, microalgae investments have reached over \$900 million worldwide [64].

Several achievements are being reported from various sources. For instance, the OriginOil algae (<http://www.oilgae.com/>) company published breakthroughs in algal biomass processing which involves a single-step extraction (Fig. 4), in contrast to three-step processing in conventional approach (Fig. 4, Right). In this a single-step extraction approach, the biomass harvesting, concentrating and oil extracting, and also separation of oil, water and biomass is run in one step in less than an hour. Smith-Baendorf [137] identified various actions to be taken in solving feasibility problems (Table 4). Siobhan [138] reported that aircraft runs on algal biofuel, which is a big promise/feedback to efforts made in the realm of algal research. It was also noted that the use of algal biofuel made the aircraft 10% more efficient and fuel consumption was 1.5 l per hour lower when compared to conventional

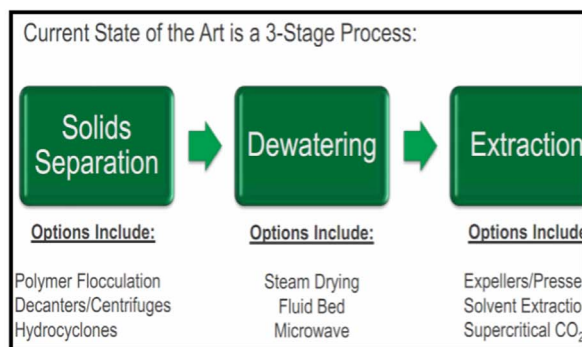
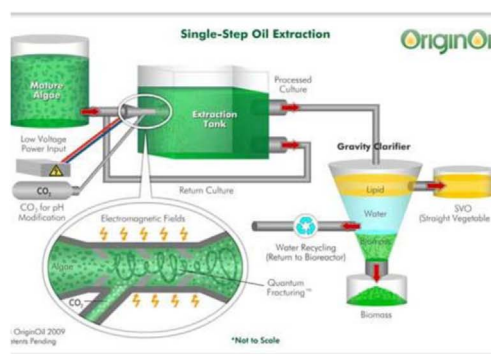


Fig. 4. A single-step extraction (left) and conventional three-step processing (right). Source: (<http://www.oilgae.com/algae/oil/extract/extract.htm>) (requested the source owner by e-mail).

Table 4

Solutions proposed to issues challenging microalgae-to-biofuel industries.

Issue	Potential solutions
Light	Pulsing light-emitting diode (LEDs), microlensed PBRs
Temperature	IR glass/plastic, use extremophilic species, using cooling water
Nutrient	Wastewater, utilize flue
Dewatering	Filamentous species, self-flocculating/settling species
Oil extraction	Genetic Modifications of excretory pathways
Costs	Co-production of a valuable product, novel PBR designs

fuel, which may be attributed to the relative high calorie content per liter of algal biofuel.

Although significant advances have been made in algal research and these have increased their industrial relevance, the application is still facing various challenges. Yet, significant scientific and engineering challenges remain in efforts geared to full commercialization of microalgae-to-biofuel technology [139]. Once the biomass is produced, the next challenge is the dilute nature of microalgal culture, which costs energy for dewatering. The finding of Danquah et al. [140] on tangential flow filtration that concentrates up to 148 times by consuming 2.06 kW h m^{-3} of energy reported to be better when compared to other methods. The latest finding reported by Matos et al. [141] that combine electro-coagulation with centrifugation proved to decrease the energy demand over >97% efficiency. Nevertheless, still much lower energy consuming techniques are demanding. Furthermore, extracting and purifying oil from algae continues to be a significant challenge in producing both microalgal bioproducts and biofuel, as microbial oil extraction is relatively energy-intensive and costly [142]. In this respect, developing economical and vigorous oil extraction and purification processes is a major challenge facing the microalgae to biofuel industries.

6. Conclusions

Microalgal biofuels are promising to replace fossil fuels in light of microalgae's intrinsic efficiency to convert solar energy into chemical energy, and their significantly higher potential yield of oils suitable for biofuel production than terrestrial crops. A number of achievements in genetic and metabolic engineering of algal strains to optimize their production costs have been recoded during the last few years. In addition, many technical aspects have been upgraded in the course of algal biomass production and biomass processing techniques to yield biofuels. Yet, the production cost is still challenging the efforts of algae-to-biofuel industries. Nevertheless, owing to the current growing concern over fossil fuels, energy security, greenhouse gas emissions, and the potential of other biofuel feedstocks to compete for limited agricultural resources, there is mounting worldwide interest in microalgal biofuels. Therefore, employing transgenic strains, accompanied by efficient harvesting and oil extracting mechanisms, combined with producing high-value products and using residual byproducts to boost production economics, microalgal production can be scaled-up to industrial level. Furthermore, the availability of plenty of sunshine and favorably high temperature in the tropics, may favor the production of hydrogen in a low-cost system in a closed photobioreactor. This would be a big opportunity especially for landlocked countries like Ethiopia, which are yet without petroleum-based oil, where there are “thirteen months of sunshine”, to boost the economy.

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