SOLAR BIOFUELS PRODUCTION WITH MICROALGAE

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With impending climate change and ever decreasing supplies of easily extractable fossil fuel, means to produce renewable and sustainable replacement fuels are being sought. Plants or algae appear ideal since they can use sunlight to fix CO₂ into usable fuel or fuel feedstocks. However, as the world population approaches the 10¹⁰ (10 billion) mark, the use of agricultural land to produce fuel instead of food cannot be justified. Microalgal biofuel production is under intense investigation due to its promise as a sustainable, renewable biofuel that can be produced using non-arable land and brackish or non-potable water. Some species accumulate high levels of TAGs (triacylglycerols) that can be converted to fatty acid esters suitable as replacement diesel fuels. However, there are many technical barriers to the practical application of microalgae for biofuel production and thus a number of significant challenges need to be met before microalgal biodiesel production becomes a practical reality. These include developing cost-effective cultivation strategies, low energy requiring harvesting technologies, and energy efficient and sustainable lipid conversion technologies. The large culture volumes that will be necessary dictate that the necessary nutrients come from wastewaters, such as the effluents from secondary treatment of sewage. Economical and energy sparing harvesting will require the development of novel flocculation or floatation strategies and new methods of oil extraction/catalysis that avoid the extensive use of solvents. Recent advances in these critical areas are reviewed and some of the possible strategies for moving forward are outlined.

Keywords: biofuels; microalgae; pyrolysis; biodiesel; hydrogen; methane digestion

1. Introduction

Microalgae are increasingly being proffered as a tenable solution to finite fossil fuel supplies. Although an upswing in new technologies has increased the potential for capturing previously unavailable oil reserves, the impact of continued greenhouse gas emissions remains. Ever increasing commercialization efforts for alternative fuel production continues to gather momentum. Greatly expanded biofuels production is being touted as an at least partial solution to the twin problems of climate change driven by excessive fossil fuel combustion and the dwindling supply of evermore difficultly extracted fossil fuels. Of course, no matter the actual biofuel, or the specific process used to make it, the ultimate energy source behind biofuels is solar irradiation driven photosynthesis.

Although several biofuels, notably bioethanol and biodiesel, are currently being produced in significant industrial quantities, 6.9×10^8 barrels in 2011 [1], there is an urgent need to develop other biofuels for a number of reasons. First generation biofuels are seen as competing with food production for resources; land, water and nutrients [2-11]. Moreover, these fuels, in particular ethanol from corn, have only a very small net energy gain (positive EROI, see Table 1 for nomenclature used in this review).

Table 1. Nomenclature

<i>AD</i>	Anaerobic Digestion, the conversion of organic matter to methane
<i>CAPEX</i>	Capital expenditures
<i>EROI</i>	Energy Return on Investment. The net amount of energy generated taking into account the various energy inputs. Also termed NER.
<i>LCA</i>	Life Cycle Analysis. The analysis of a production process that takes into account the various impacts from “well to wheel”
<i>OPEX</i>	Operational Expenditures
<i>PAR</i>	Photosynthetically Active Radiation (Light in the visible range that is effectively converted by photosynthesis, 400-700nm)

PE Photosynthetic Efficiency, the efficiency of conversion of light energy to chemical energy

Finally, given the constraints on land-based plant productivities, enormous surface areas would be required to make significant impacts in terms of reductions in fossil fuel use. For example, to replace the amount of oil used in the US transportation sector in 2014, 4474 million BOE [12] with biofuels would require $19.3 \times 10^6 \text{ km}^2$ if soy beans were used (40 gallons/acre) or $1.93 \times 10^6 \text{ km}^2$ for corn (400 gallons/acre), clearly impossible since the arable land available in the US is $1.5 \times 10^6 \text{ km}^2$ [13]. On the other hand, biofuels produced from microalgae hold the promise of a much small footprint. Although actual large scale production figures are not available, a rough approximation can be made using reasonable extrapolations from existing data. Thus if a microalgal production system could produce $20 \text{ g m}^{-2} \text{ d}^{-1}$, and 30% of this could be converted to liquid fuel, the land area required for total transportation fuel replacement would be $4.47 \times 10^5 \text{ km}^2$. As well, the International Institute for Sustainable Development has concluded that the benefits to climate and CO_2 reduction from replacing petroleum fuels with biofuels like ethanol are essentially zero [6]. Thus, more advanced biofuels are needed that can meet these challenges.

Microalgae, small, unicellular organisms that carry out plant type photosynthesis, possess several characteristics that make them attractive as potential biofuels producers [7-10]. Their autotrophic capabilities lend an element of flexibility in comparison to conventional fuels. As discussed in more detail below, algae can serve as feedstocks in a variety of processes that can produce fuels or fuel precursors (Figure 1).

Figure 1. Overview of Solar Biofuels Production by Microalgae

Microalgae supported by basic nutrients, use captured solar energy and plant-type photosynthesis to split water and fix carbon dioxide. A variety of fuels can be made from the newly fixed carbon. The harvested algal biomass can be converted to bio-oil through hydrothermal liquefaction or pyrolysis or to methane through anaerobic digestion. Alternately, different algal storage materials can be converted to various fuels including ethanol, biodiesel, and hydrogen. Metabolic engineering could be used to enhance yields or to produce novel fuel compounds.

Microalgae are considered to have great potential due to; (1) their ability to be grown on marginal lands, (2), their use of wastewater as a medium for growth, often thereby effecting wastewater treatment as well, (3) their very fast growth rates as compared to land plants, often

doubling their biomass in a single day or less, and (4) their ability to sequester flue gas emissions under appropriate conditions [14]. Depending on the production methods used, microalgae can provide a suite of commercially useful co-products [15-17]. In addition to triglyceride production for biofuels, the co-production of other even more valuable compounds, such as essential fatty acids and animal feeds, could bolster the economics of the whole process at the beginning when fuel production is modest. However, scale up and production costs must be considered to truly ascertain commodity values under present market conditions [18]. In some instances, when biofuel production is ramped up to scale, these valuable co-products could lose market value.

Microalgae carry out plant type photosynthesis, converting captured solar energy into chemical energy through the fixation of CO₂. Although only specific products are naturally made, environmental, biochemical and molecular manipulations potentially greatly expand the scope, leading to practically limitless possibilities. A variety of biofuels are possible through different processes. Total algal biomass can be converted through direct thermochemical transformation to bio-oil, or through anaerobic digestion, to methane. Pyrolysis treatment under varying temperature regimens appears to have some potential for oil production. According to some, oil yields may range from 20-50 % of the total ash free dry weight in some algae [19]. Alternatively, algae can be induced to produce high levels of storage compounds, starch or lipids, which can, when appropriately extracted and converted, be turned into the biofuels, bioethanol or biodiesel. A variety of novel methods for extraction and conversion remain to be explored including the use of enzymes to aid in extraction by weakening the cell wall [20]. Finally, future metabolic engineering [21-24] could produce improved strains with the ability to

produce higher levels of natural biofuels, or endowed with the capacity to produce novel fuels, or fuel precursors, such as ethylene, biobutanol, hydrogen, or biojet fuel.

However, there are a number of serious challenges to implementation of microalgal-based biofuels production on a practical level [25]. These range from uncertainties on how to effectively and efficiently culture algae on a large scale, how to maintain the desired culture in the face of alien species, how to carry out low-cost, energy efficient harvesting, and the best methods for conversion to biofuels. In what follows, different aspects of algal culture and harvesting are briefly discussed and the various options for making fuels from algal biomass are presented in some detail.

2. Algal biofuels; processes and products

2.1 Limits to photosynthesis

Of course any process that relies on natural plant-type photosynthesis for solar energy conversion to various compounds or fuels will inevitably be limited by the inherent inefficiencies

of photosynthesis (Figure 2).

Figure 2. Solar Energy Train-the Limits of Photosynthesis

The various physical-chemical and biological processes that lead to energy loss are shown on the left, and on the right the effect on the limits of photosynthesis are given in terms of percent energy left.

The limiting factors are well known and have recently been discussed in detail [9, 10, 26, 27].

Less than half of the incoming radiation (45%) is actually able to be captured by the photosynthetic apparatus, some (about 10 %) gets reflected from the various biological structures, and there are losses (21%) during transfer from the antenna complexes to the reaction centers. A large fraction (65%) of the remaining 32.8% of incoming energy gets lost during transfer to chemical energy, much of this is in fact necessary in order to provide thermodynamic

driving forces at the various steps. Finally, not all the energy can be used, especially at high light intensities and some of the newly made chemical energy is lost, burned during dark periods to maintain cell function (20%), or to consume potentially damaging excess oxygen and to maintain cells during darkness (40%). This leaves only about 5.5% of incoming solar flux as the maximum of energy that can be finally converted into cell biomass or product.

These factors affect any process that relies on plant-type photosynthesis and in many ways fix the absolute amount of energy that can be obtained in this way since very little can be done to affect the various losses. Small gains might be possible through some kind of bioengineering, to extend the range of light harvesting for example, or possibly to decrease losses during transfer to the reaction centers or during conversion to chemical energy. How much might be gained in this way is debatable since in fact natural evolution has had a few billion years to perfect this process.

2.2 Algal cultivation and harvesting

These aspects of algal biofuels have been recently reviewed in detail [7-10] and thus are only briefly presented here. These processes have been extensively examined, but, as yet, there is no consensus view as to the best way forward. Some of the issues are shown in Table 2.

Table 2. Issues in algal culture and harvesting

Concern	One option	Another option
Organism	Specific monoculture	Local species, Mixed culture
Facility	Enclosed photobioreactor	Open, raceway pond
Growth Medium	Defined, N and P inputs	Wastewater
Harvesting	Centrifuging (High energy)	Flocculation, settling, dissolved air flotation

Of course, here how they will be cultivated will also determine what is possible in terms of algal culture. As indicated in Table 2, some propose the use of well characterized strains since these have been shown to possess some desirable characteristics, i.e. high lipid yields. However, these may not be competitive in open ponding operations which are probably more suitable for locally selected strains or even mixed cultures, which might even have certain beneficial characteristics [28]. Enclosed photobioreactors might permit the use of well characterized select strains since contamination can be kept to a minimum and this type of cultivation system is much more amenable to process control. Open ponds and photobioreactors each have their advantages and disadvantages, as shown in some detail in Table 3, and, in fact, the suitability of a particular process depends upon the desired application. Photobioreactors, in addition to offering the ability to more closely control the microalgal growth environment, appear to be inherently more productive on a volumetric basis (Table 3).

Table 3: Pros and cons of photobioreactors and open ponds

Issue	Open ponds	Photobioreactor	Reference
Control of pH, temp, dissolved CO ₂	Difficult	Easy	[29-31]
Ease of contamination	High	Low	[29-31]
Water evaporation	High $\geq 10 \text{ L m}^{-2} \text{ d}^{-1}$	Low $\geq 2 \text{ L m}^{-2} \text{ d}^{-1}$ (cooling)	[32]
Areal Productivity (m ⁻²)	Medium	Medium	[32-34]
Volumetric Productivity	Medium 0.07-0.18 g DW L ⁻¹ d ⁻¹	High 0.4-1.9 g DW L ⁻¹ d ⁻¹	
OPEX	Low	High	[30, 31, 35]
CAPEX	Low	High	
EROI	Medium 8.3	Low 0.2-4.5	[32, 36, 37]

However, what counts here most is areal productivity, which determines the land area required, and on that basis open ponds and photobioreactors seem to have about equal performance [32-34]. Most would agree that photobioreactors cannot be used for biofuel production since the CAPEX (capital expenses) and OPEX (operating expenses) associated with them are much higher than for open ponds (~3-5X), making them uneconomical for such a low value product. This is a consequence of the relatively diffuse energy content of incident solar radiation. A simple calculation based solely on incident light intensities and assumed photosynthetic conversion efficiencies and fuel prices shows in fact that the economic constraints are formidable (Box 1).

Box 1: Solar energy is diffuse, its energy content is low!!

At a very favorable location:

$$5 \text{ kWh m}^{-2} \text{ day}^{-1} = 6.6 \text{ GJ m}^{-2} \text{ year}^{-1} = \mathbf{1\text{BOE m}^{-2} \text{ year}^{-1}}$$

Under optimistic assumptions:

- 10% conversion efficiency
- \$100 per BOE

$$\mathbf{\$10 \text{ m}^{-2} \text{ y}^{-1}}$$

Under more realistic assumptions:

- 1% conversion efficiency
- \$50 BOE

$$\mathbf{\$0.50 \text{ m}^{-2} \text{ y}^{-1}}$$

In addition to economic constraints, any proposed biofuel process must have a positive EROI, i.e. produce more energy than is consumed. In this regard as well open ponds fare better than photobioreactors. One study compared, on a theoretical level, open ponds, flat-plate photobioreactors and tubular

reactors, producing 100, 000 kgs of algal biomass per year and found that the EROI was 8.3, 4.5, and 0.2 respectively [38]. Likewise, a metastudy of seven recent LCA studies concluded that in almost all cases open ponds showed a positive EROI whereas the photobioreactors had a largely negative EROI [36].

As well, although almost all laboratory studies reporting high growth rates and/or high lipid yields have been carried out using defined synthetic media, this would be impractical for large scale algal production for a number of reasons. One obvious negative is that nutrient addition in this manner could be quite costly at large scale. Even more significant is the large pressure that this would put on nitrogen and phosphate based fertilizers, essential for high-yielding food production. This would thus conceivably bring algae-based biofuels production in competition with food production. Fortunately, microalgae in general are known to thrive on a variety of wastewaters, in particular secondary effluents from sewage treatment plants. The use of these waters is triply beneficial; no potentially scarce freshwater needs to be used, the required nutrients are readily obtained from the wastewater, and finally, algal growth will carry out additional wastewater treatment [3, 5, 39]. Thus any truly large scale algal biofuels production facility should and probably would be built where suitable wastewaters are readily available. In fact, water use is a real issue as well in most cases since it has been estimated that each liter of algal biodiesel requires the use of 3000 liters of water [3, 5, 40]. Obviously recycling the water from algal cultivation is a necessity.

Finally, no matter how dense the algal growth is, they will need to be removed from a medium that is at least 99% water. Given their small size, this can present a formidable challenge. Although strategies exploiting biofilm formation on harvesting apparatuses are being investigated [40], most production methods require dewatering of microalgae grown in suspension [41]. Common laboratory practice, centrifugation, is of course probably out of the question considering the significant energy input required by this process, contributing to a very poor EROI. Therefore, low cost alternatives, natural settling, or flocculation assisted settling, must be used in at least the initial step with perhaps centrifugation for final dewatering of the

now concentrated algal slurry. It might also be possible to develop a suitable membrane process for this final processing step [42-43]. A number of options are currently being explored for effective flocculation, including the use of electrical input or filamentous fungi [44-48]. Use of some variation of dissolved air flotation is promising since this technique has already proven effective in water treatment processes at removing microalgae and is relatively indifferent to species composition. The challenge is to develop a variation requiring minimal energy input [49, 50]. Addition of flocculating agents and detergents can increase the efficiency of this process [51].

Detailed well-to-wheel EROI and LCA analyses are absolutely necessary before a particular biofuels production process can be adopted in a rational manner [37]. Part of the problem with first generation biofuels is that they were often adopted through political expediency without thorough analyses of the real energy inputs and the extended environmental impacts. In order to keep costs, both economic and energetic low, some form of dissolved air flotation or settling [41] could be good options. Settling can either take the form of selection for species with a natural tendency to settle in unmixed solutions, and/or can be further aided through the use of some sort of flocculation process.

Thus, although there is a great deal of promise in the use of microalgae for biofuels production, there are a number of significant challenges that must be overcome before this can become a practical reality. A great deal of R&D in this area is ongoing, and the next few years may well see some advances in these areas. In what follows, the various options for fuel production from harvested biomass are examined.

2.3 Thermochemical transformation

One possibility is to convert the entire harvested algal biomass to some type of fuel using a thermochemical process [52-61]. This is also a useful option for treating algal residues after some extraction process, for example after lipids have been extracted for biodiesel production, thus increasing total energy recovery [62]. These processes are promising since the whole algal biomass, not just the lipids or carbohydrates, can be converted into a fuel, thus special cultivation conditions, such as nitrogen deprivation to increase lipid content, are not required.

Table 4. Characteristics of different thermochemical transformations

Thermal process	Conditions	Products
<i>Gasification</i>	900 °C, partially aerobic	Syngas (H ₂ , CO)
<i>Pyrolysis</i>		
slow	400 °C, 30 min ret time	35% char, 30% liquid, 35% gas
fast	500 °C, 10-20 s ret time	20% char, 50% liquid, 30% gas
flash	500 °C, 1 s ret time	2% char, 75% liquid, 13% gas
<i>Hydrothermal</i>		
carbonization	200 °C	Lipid soaked biochar
liquefaction	350 °C	Biocrude
gasification	600 °C	Gases (H ₂ , CH ₄)

Several options are available with the possibility of making different fuel molecules or precursors. Applying high temperatures to dry biomass in the partial or complete absence of oxygen, processes called gasification and pyrolysis respectively, transforms the biomass into a mixture of solids, liquids and gases. In a gasification reaction, high temperatures and limited amounts of oxygen are used, in conjunction with a catalyst, to decompose the various biomolecules to hydrogen and carbon monoxide, a mixture referred to as syngas. This gas mixture can either be directly combusted, or used to synthesize other, higher grade, fuels. Pyrolysis produces a mixture of biochar, bio-oil and gases (methane, hydrogen, carbon monoxide and carbon dioxide) whose relative abundance is affected by the temperature used, and hence

requires at a minimum additional separation steps and upgrading reactions. While pyrolysis is characterized by a relatively low capital cost and relatively high biofuel yield, it suffers from the requirement for dry biomass and in general, the biofuel obtained is of rather poor fuel quality. In fact, both these processes have been little studied with respect to microalgal biomass, principally due to the requirement that the biomass be dry, incurring appreciable costs in the case of microalgae.

Alternatively, wet biomass can be used in processes that are called hydrothermal liquefaction (HTL) and catalytic hydrothermal gasification (CHG) that take place at moderate temperatures under pressures where water is close to its supercritical state. Thus lower temperatures, but higher pressures are used than is the case for pyrolysis, incurring higher capital costs.

Hydrothermal liquefaction has the advantages that wet algal biomass can be processed and mass transfer is enhanced with supercritical water as reaction medium. In HTL and CHG, catalysts are used to accelerate the reaction and to control product distribution. On the other hand, this raises the challenges of catalyst recovery and catalyst inactivation over time. Temperature, total solids, and retention time are factors that affect bio-oil yields and quality, with one study showing an inverse relationship of temperature on these two process parameters [59].

The liquid fraction from both HTL and pyrolysis, often called bio-oil or biocrude, is in fact a complex mixture of different organic compounds, and, depending upon the exact process as well as feedstock, may require additional fractionation and upgrading to produce a drop-in fuel. One of the major challenges in producing fuels from algae in this manner is the high nitrogen content of microalgae. If left in the fuel, this N would lead to unacceptable levels of NO_x when combusted so it must be removed during the upgrading process. In addition, in many cases the relatively high oxygen and sulfur contents of bio-crude would also be problematic if used

directly in combustion. Fractionation of complex biocrude yields a mixture of gasoline, diesel, and heavy oil, which in one case were 25%, 50%, and 25% respectively [57].

Catalytic hydrothermal gasification (CHG) using supercritical water produces primarily a gas phase consisting of variable amounts of hydrogen, carbon dioxide, carbon monoxide, and methane [56]. However, this process, as presently practiced, presents a number of challenges, including the production of tars, catalyst poisoning by the sulfur that is present, and the requirement for relatively high temperatures (350-500 °C) and pressures (18-36 MPa). A combined continuous system, where HTL is used to convert the main incoming biomass and CHG is used to treat the process water, recovering additional energy, has been estimated to give a total conversion cost of \$1.18 GGE (gallon of gasoline equivalent) [63, 64].

2.4 Anaerobic digestion

A variety of biological means are available for transforming microalgal biomass or its

constituents to fuel compounds. Perhaps the least technically challenging is the use of anaerobic digestion which uses a host of different microbes with a wide variety of metabolic activities to convert complex organic substrates into methane and carbon dioxide (Figure 3).

Conversion of various substrates to methane proceeds through a series of steps carried out by different organisms with differing metabolic capacities. Initial depolymerization reactions free

monomeric substrates and then coupled metabolisms are active in which the products of one type of organism serve as the substrates for the next, eventually leading to the production of methane and carbon dioxide. Since these reactions are carried out by a community of organisms, the combined degradative capacity is large and a wide variety of substrates can therefore be converted to methane. This offers the potential for conversion of the different classes of compounds in an algal cell into methane.

Anaerobic digestion is ubiquitous in nature, and is responsible for a large amount of recycling of fixed carbon each year (350-700 Tg/year) as well as the majority of natural gas deposits of fossil methane. Thus, this process offers the possibility to convert algal biomass, or algal biomass residues into methane [65, 66], a fuel (55 MJ/kg) already in large use in stationary applications but also adapted for transportation use, often in buses, as LNG (liquefied natural gas). Thus this process could be used directly with harvested biomass to convert this into useable energy, but is perhaps even more useful for converting algal residues that are left over after molecules that can be converted into even more useful fuels (biodiesel, bioethanol) have been extracted [66, 67]. Not only does this increase the overall economics of the process, it effectively extracts much of the nutrients from the harvested algal biomass (nitrogen, phosphate, trace elements) allowing their return into the cultivation system. Pretreatments (thermal, ultrasonic, and alkali) to augment solubilization of the algal biomass have been investigated in an effort to increase efficiencies for methane production. However, at least some reports suggest that only very modest gains in net energy can be realized in this way, suggesting that the direct use of algal biomass is sufficient for significant methane production, probably due to the inherently strong solubilization activities of the microbial community that is present [68]. One study which examined under the same conditions a number of different strains as well as mixed

cultures found a wide variation in actual methane yields; from 0.161 to 0.435 L CH₄ g⁻¹ VS representing a wide range in the conversion of the calorific value of the biomass (from 26 to 79%) [69]. Of course some of this is due to differing composition, i.e. differences in lipid, carbohydrate and protein content, between different strains, but in general algal biomass has a suboptimal composition for methane digestion since with its relatively high nitrogen content it falls outside of the optimal C/N for methane digestion, which is in the range 20-30. One solution is to carry out co-digestion, preferably with substrates that will add additional carbon, such as paper [70, 71]. If algal ponding operations are carried out in conjunction with wastewater treatment, an ideal situation in at least some respects as already discussed, then it makes sense to carry out a co-digestion of algal biomass with the activated sludge from the secondary wastewater treatment process [71]. Of course, the downside, and the reason that methane is really an orphan biofuel, is that it is a gas and not very readily adapted to the present day transportation infrastructure which by and large demands liquid fuels.

2.5 Biofuels derived from storage compounds

Many different biofuels can be derived in principle from the various storage compounds that diverse algae are capable of making. Having evolved in environments with fluctuating nutrient supplies, many microalgae are endowed with the capacity to continue to fix carbon dioxide and store the excess fixed carbon when growth is arrested for some reason, for example nitrogen limitation.

2.5.1 Starch as reserve material

Many algae store fixed carbon as some form of carbohydrate, most commonly starch, but some make glycogen. These are easily mobilized as glucose when the need arises. Likewise, these compounds can be extracted from the harvested algal biomass, cultivated under conditions that

have maximized starch formation, and converted to sugars to feed some form of fermentation. In essence then, the starch-derived sugars can replace corn or sugar cane derived sugars in microbial fermentation processes leading to bioethanol or biobutanol production.

Among the many potential advantages are that, as unicellular organisms, microalgae contain the desired feedstock in every cell, and hence there are no auxiliary materials, such as roots or stalks, that become residues that have taken some of the converted solar energy to make but which are not used in fuel production. This in itself inherently increases efficiency. Microalgae, depending upon the species and cultivation conditions, can be up to about 50% starch by weight [72], quite often accumulating in the plastid (chloroplast) but sometimes in the cytosol [73]. Interestingly, one study using *Chlorella sorokiniana*, found that not only did growth under higher $p\text{CO}_2$ (CO_2 partial pressure) increase biomass, as commonly observed, but it also changed the location of the accumulated starch, from the plastid to the stroma, as well as its structure, making it more easily deconstructed [74]. Various factors, including nutrient limitation, can increase starch accumulation, and the accumulated starch can in principle be either be directly fermented by endogenous metabolism to ethanol [75], or be extracted and converted to sugars [76] followed by a more conventional yeast fermentation.

2.5.2 Lipid (TAGs) as reserve material

Microalgae are widely known for their propensity to accumulate large amounts (up to 80% by dry weight) of neutral lipids that can be converted to biodiesel [7-10, 26, 27]. While special conditions are probably required to get an extremely high lipid content, strains can easily be isolated that achieve lipid levels of 30 to 40% on a dry weight basis without extensive manipulation [77, 78]. This process is under extensive R&D with over 100 start-up companies formed and over one billion invested over the past decade. Although some of this frenzy has

been caused by overly optimistic claims, there are a number of attractive features to such a process, not the least of which is that, depending upon the species, a biodiesel with drop-in fuel characteristics can be made.

Of course, there is still much debate about the best way to develop a practical process out of this system [79]. In fact, there are unresolved issues surrounding each of the various stages of liquid (biodiesel) production using microalgae. It is not even evident how they should be cultivated at large scale given the number of significant challenges. This issue was already raised in section 2.2 but further discussion here is warranted since this remains a very active area of discussion for this biofuels pathway. Two basic methods have been proposed, enclosed photobioreactors and open raceway ponds (Table 3). In terms of bioprocess control and maintenance of the desired species, enclosed photobioreactors obviously have the upper hand. Although it would hardly be practical at scale, specialized bioreactors can be used to increase the effective use of solar irradiance. For example, the use of outdoor flat solar panels with sun tracking capabilities can improve biomass accumulation through photoinhibition reduction and improved temperature control [80]. On the other hand, it would seem that the large differences in anticipated CAPEX and OPEX would mean that only open raceway ponds will make practical economic sense for low value products such as fuels. Additional factors to consider include the fact that at scale enormous quantities of water and nutrients will be required. Although in some locales and some situations the use of amended sea water might work, in general advantage needs to be taken of the enormous supplies of wastewater, primarily from sewage treatment, that are available worldwide [7, 8, 81, 82]. The use of wastewater should help reduce the overall costs of microalgae cultivation, reducing potentially expensive nutrient addition as well as obtaining credits for wastewater treatment. Many strains of algae grow quite happily in such a

medium with little or no supplementation and, additionally, some can be quite effective at removing remaining nitrate and phosphate from these waters [77, 78, 83, 84].

One of the major challenges in the algae to biodiesel process is to find that produces high yields of biodiesel without requiring large amounts of energy or the use of toxic solvents. Thus, different novel extraction and conversion procedures are under active investigation [85-86]. One possibility is to use switchable solvents which can be cycled between a polar and a nonpolar character simply by using CO₂, eliminating the need for solvent recovery through distillation [88]. Equally promising is the recent demonstration of a wet lipid extraction procedure allowing relatively high (80%) recovery of lipids from wet algal biomass (84% moisture content) [89-93].

Although practical systems capable of delivering biodiesel derived from algae at prices competitive at the pump with fossil diesel are not yet there, production is ramping up in attempts to meet various government mandates, including the US Navy's Green Fleet initiative [94]. Given the large amount of activity and interest in this area of biofuels production there will soon be a number of pilot plants of significant size in operation and data on real world operations will start to accumulate. Practicality will depend upon how well engineering and science can overcome the challenges as this process scales to significant size.

2.6 Hydrogen production by algae

Hydrogen has many desirable characteristics as a fuel, including the highest gravimetric energy density (142 MJ/kg), the ability to be converted efficiently into electricity through the use of fuel cells (94% compared to a maximum of about 60% for internal combustion engines), and having very clean combustion products (H₂O). For many years, researchers have been pursuing the dream of making hydrogen by using water splitting catalyzed by biological photosynthesis. As has been known for many years, some microalgae contain the enzyme hydrogenase and are

capable of using it to reduce protons to hydrogen when re-illuminated after a period of dark adaptation during which the culture has become anaerobic. The problem is that such a reaction is normally short-lived since the newly generated oxygen quickly inactivates the hydrogenase enzyme which is extremely sensitive to oxygen [95, 96].

Therefore, to realize the dream of generating hydrogen from the almost limitless substrate, water, by capturing solar energy through photosynthesis, this serious challenge must somehow be overcome. A number of strategies are being applied in attempts to circumvent this oxygen problem. On the one hand, nutrient deprivation, in particular sulfur, has been shown to lead to prolonged periods of hydrogen evolution. However, this occurs because total photosynthesis is decreased to the extent that respiration with the supplied acetate can soak up all the oxygen that is evolved through photosynthesis. This is not practical for two reasons [96]. Photosynthetic efficiencies are already low (Figure 2) so decreasing this further is a step in the wrong direction. Furthermore, use of a fixed carbon substrate also represents a loss in photosynthetic efficiency since this also is a product at some point of photosynthesis.

Another strategy is to develop oxygen tolerant hydrogenases [95, 96], but in reality this is a daunting task given the very small sizes involved of both the poisoning oxygen and the product, hydrogen. Oxygen is toxic due to its attack on the highly reactive active site, a complex arrangement of iron, sulfur, and ligands like CO and CN. It is unclear how to prevent access of O₂ to this site, buried within the protein, while at the same time allowing the H₂ that is generated to escape. Likewise, it is unclear how one would change the active site to make it less reactive to oxygen yet still have high activity towards proton reduction.

2.7 Metabolic engineering

Finally, metabolic engineering could be applied to solar biofuels production in a number of ways. Different aspects could conceivably be changed to make this a more efficient process [96-101]. These improvements could include extending the range of light capture and making photosynthesis more efficient at high light intensities by reducing the size of the photosynthetic antennae. In another approach, metabolic alterations could be carried out to channel more of the captured energy towards production of the desired product by, for example, eliminating competing pathways, or increasing the amounts and activity of the enzymes involved in product formation [102-104]. As more information on the regulation of lipid production is gleaned from the analysis of transcriptomes and lipidomes [105, 106], additional targets for metabolic engineering may be revealed. Meanwhile, the molecular toolkit for the genetic engineering of microalgae is growing ever larger and more sophisticated, with applications in production strains such as *Nannochloropsis oculata* and *Phaeodactylum tricornutum* as well as model organisms such as *Chlamydomonas reinhardtii* [107-109]. Finally, the quality or type of fuel could be changed through the application of these strategies and techniques. Methods pioneered to enhance production of commercially important nutraceuticals like omega-3 long chain polyunsaturated fatty acids could similarly be developed to optimize biofuel production [110, 111]. For example, fatty acid chain length or saturation could be tailored so that a fuel, biodiesel or biojet, with the desired characteristics is made [112]. It is even possible to envisage in the future algae endowed with new pathways capable of producing chemicals and fuels, such as ethylene, not possible with native cells.

3.0 Conclusion

Algae seem uniquely poised to enable the production of solar biofuels. These, in theory, can be made at high productivities without competing with food production. Additional benefits could

be wastewater treatment and the development of biofertilizers. Nevertheless, a number of significant technical challenges prevent a large scale practical implementation of algal technologies for fuel production at present. Research and development work currently underway in a large number of academic and private enterprise laboratories worldwide promises to provide solutions to many of these problems in the short to medium term.

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