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Algal biofuels: Challenges and opportunities

Gustavo B. Leite, Ahmed E.M. Abdelaziz, Patrick C. Hallenbeck *

Département de microbiologie et immunologie, Université de Montréal, CP 6128, Centre-ville, Montréal, Canada PQ H3C 3J7

HIGHLIGHTS

- Algae are promising for biofuels production.
- Higher productivity and lipid content than plants.
- Open ponds are better than PBRs for biofuels.
- Technical hurdles include harvesting and oil extraction.

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ABSTRACT

Biodiesel production using microalgae is attractive in a number of respects. Here a number of pros and cons to using microalgae for biofuels production are reviewed. Algal cultivation can be carried out using non-arable land and non-potable water with simple nutrient supply. In addition, algal biomass productivities are much higher than those of vascular plants and the extractable content of lipids that can be usefully converted to biodiesel, triacylglycerols (TAGs) can be much higher than that of the oil seeds now used for first generation biodiesel. On the other hand, practical, cost-effective production of biofuels from microalgae requires that a number of obstacles be overcome. These include the development of low-cost, effective growth systems, efficient and energy saving harvesting techniques, and methods for oil extraction and conversion that are environmentally benign and cost-effective. Promising recent advances in these areas are highlighted.

1. Introduction

The transportation sector plays a major role in the production of greenhouse gas (GHG) emissions, as well as being responsible for 28% of total world primary energy consumption, mainly consisting of fossil fuels, and for 71% of the total crude oil used (Energy, 2004; Pienkos and Darzins, 2009). Transportation fuels can be divided into three groups related to use: private vehicles (gasoline); commercial vehicles and stationary engines (diesel); or jet fuels (kerosene). World consumption of diesel was nearly 1460 trillion liters in 2011 (OPEC). Fuel demand in the transportation sector is projected to increase by 40% over the period 2010–2040 (ExxonMobil, 2013). Most of this demand is driven by the commercial sector with heavy duty vehicle (diesel) fuel use increasing by 65%. Although the number of light-duty vehicles (cars) could double, the increased fuel demand might be largely offset by increased fuel efficiency and the switch to hybrid technologies (ExxonMobil, 2013).

Any plan to lower GHG emissions will require the substitution of at least part of the petroleum-based fuels used for transportation. Today we “*borrow land from the past*” (Wackernagel and Yount, 1998), by using carbon which was fixed in another era. Even at present prices, crude oil is cheap, easily extracted and easy to use since it just needs to be taken from its natural reservoir and distilled into products. However, its use reintroduces into the atmosphere carbon trapped millions of years ago. In addition to the role of fossil fuel combustion in climate change due to the increased concentration of CO_2 in the atmosphere, a well established mathematical model used to calculate crude oil field reserves and production capabilities predicts peak oil within the next few decades (Nashawi et al., 2009).

After a hundred years of intensive use, humanity has become strongly dependent on fossil fuels, we are addicted to oil. The world’s economy relies on the very efficient system of production, distribution and use that has been developed. Any transition to a new fuel will have to be “painless”, using the technology and infrastructure of the existing system as much as possible. The first generation of biofuels fit this model as bioethanol and biodiesel require minimal or no adjustment of regular internal combustion engines, and can generally be distributed, stored and pumped like conventional crude oil-derived fuels. The major drawback to the use of these alternative fuels is that arable land is used to farm the corn, sugar cane or oil seed

* Corresponding author. Tel.: +1 514 343 6278; fax: +1 514 343 5701.
E-mail address: patrick.hallenbeck@umontreal.ca (P.C. Hallenbeck).

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crops needed to produce these fuels. In addition, it would be impossible to produce the quantity of biofuels that would be necessary to meet present fuel demands using first generation technology. In 2010, the US consumed nearly 220 trillion liters of diesel (Energy Information Administration, 2012). To produce this volume of fuel using soybeans for example (average yield of 600 liter per hectare), would require 367 million hectares, in contrast with the only 178 million hectares that is currently available for cropland and the 930 million hectares of total US land area (EIA, 2012). In addition, the commodities used for first generation biofuels production have other possible markets as sugar, animal feed or cooking oil. A farmer will negotiate the selling price of his product in order to profit as much as possible, enhancing even more the competition between food and fuel and creating a complex fluctuation of food prices linked to fuel demand. With actual world production of biofuels at 109 trillion liters per year (86.6 trillion liters bioethanol, 24.4 trillion liters of biodiesel) (EIA, 2012), there has been a great deal of speculation as to whether or not this is already happening. Thus it is clear that although production of first generation biofuels was an important step, it is however only a palliative solution and is untenable in the long term.

2. Microalgae

The call for advanced biofuels demands "drop in" fuels able to be used with the existing infrastructure for storage and distribution, from manufacture to the final customer, but with a production system able to be scaled up without competing with food crops for land. Microalgal biodiesel has been proposed as the most obvious choice. Microalgae are oxygen producing microorganisms containing chlorophyll "a", mostly autotrophs, using atmospheric CO_2 as primary carbon source whereas some can grow mixotrophically, facultatively using an organic source of carbon in addition to CO_2, or even heterotrophically, using only previously fixed carbon as a carbon source. Some are obligate heterotrophs, unable to perform photosynthesis due to a defective plastid. Thus, microalgae can be pictured as single or associated cells floating in oceans, rivers or lakes and using sunlight to produce and store fixed carbon. Thousands of prokaryotic (cyanobacterial) and eukaryotic species match this description; they are the primary producers in oceans, supporting three-fourths of the planetary food chain. The ancestors of microalgae go all the way back to the origin of life and have been directly linked to past events of climate change, transforming the composition of the Earth's atmosphere by the production of O_2, and mitigating CO_2 by sinking fixed carbon deep in the ocean (Buesseler, 2012).

By the above definition, the term "algae" is an artificial way to group tens of thousands species which are in fact taxonomically distributed over several kingdoms; Protista, Chromista, and Plantae (Woese et al., 1990; Cavalier-Smith, 2004; Guiry and Guiry, 2012). These organisms inhabit the most divergent environments, with some species colonizing the Earth's poles and others causing blooms in the tropics (de Morais and Costa, 2007; Cellamare et al., 2010; Mutanda et al., 2011; Pereira et al., 2011). They are found in hyper-saline to fresh water environments, over a broad range of pHs, and even relatively dry environments such as soil and rocks. Microalgae are adapted to inhabit almost any place with enough humidity, and many are also able to enter into a dormant state until there is enough moisture to resume metabolism. They are taxonomically rooted with the ancestor of land plants, an organism formed by the endosymbiosis between a heterotrophic eukaryotic host cell and a cyanobacterium, which formed the plastid. This event is thought to have happened 1.5 billion years ago (Yoon, 2004) and subsequent differentiation and further endosymbiotic events gave rise to branches such as the green algae and the red algae.

Table 1
Conventional diesel cost as of August 2012 (retail price US-$1.05/L) {EIAUSEnergyInfo:tm}.

Diesel fuel cost	Share (%)	Value (US-$)
Taxes	12	0.126
Distribution and marketing	14	0.147
Refining	14	0.147
Crude oil	60	0.630

3. Algal biofuels

Any organism dependent on sunlight as its primary energy source needs to store energy-rich compounds to avoid starvation when light is not available. Vascular plants synthesize a variety of energy rich molecules to save enough energy from the sunlight period for a rainy day (or night). A Canadian example would be the maple tree and the phloem with its high sugar content (Maple Syrup). Vascular plants often produce oil as a carbon reserve for germination. To increase embryo viability, some plants accumulate part of the energy in the seed as TAGs (triacylglycerols), which is historically accessed by press extraction (e.g. olive oil). Microalgae are capable of the synthesis and accumulation of a variety of high energy molecules, including fatty acids (FA) and TAGs, the major feedstock for biodiesel production.

However, species with a high lipid content are not phylogenetically related, occurring in different kingdoms, Protista (e.g. Dinoflagellates), Chromista (e.g. Diatoms) and Plantae (e.g. Chlorophytes). TAG content varies among strains of the same species in quantity and quality (Leite and Hallenbeck, 2012). Nevertheless, lipid content higher than 50% is frequently described in many species, which represents one of the advantages of using microalgae instead of vascular plants for biodiesel production. Only the seeds of a vascular plant are used when making plant-derived biodiesel, with the rest of the biomass usually considered waste. Consequently, the aerial production yield of lipids from microalgae has the potential to be many times higher than that of the already developed technology of oil seed crops, with the advantage of not requiring arable land. Another key factor for choosing microalgae as a system for biodiesel production is their potentially minimal nutritional requirements. Microalgae can be grown on fresh or marine water, on marginal lands, and even in association with wastewater treatment plants or industrial parks where their cultivation offers the additional benefit of bioremediation. After the extraction of hydrocarbon for biodiesel production, the biomass can be processed in an anaerobic digester for methane production, a secondary source of energy, with the digester effluent fed back into the algae cultivation system as a source of nutrients. Even though production with such a system may not completely satisfy local fuel demands, it will evidently lower the importation of fuel, creating a decentralization of production (Table 1), improving the local economy and helping the environment.

4. Cultivation

Two basic alternatives for microalgae cultivation exist and their relative merits are the basis of ongoing debate. Some of the factors involved are listed in Table 2.

4.1. Photobioreactors

These are systems where the cultures are enclosed in some transparent recipient. Photobioreactors (PBR) can have different sizes and shapes: plastic bags, flat panels, tubes, fermenter like and others. Vertical tubes are among the most popular system

Table 2
Photobioreactors and open ponds; pros and cons.

Photobioreactor	Issue	Open ponds
Easy	Control of of culture conditions pH, temp., dissolved CO_2	Medium
Low	Susceptibility to culture contamination	High
Low	Water evaporation	High
High	Productivity per m^2	Medium
High	Energy input	Low
High	Structure cost	Low

Table 3
Photosynthetic efficiency train.[a]

Minimum energy loss	(%)	Percentage remaining (%)
Radiation outside useable range (non-PAR)%	55	45
Reflection	10	41.5
Transfer to reaction center	21	32.8
Conversion to chemical energy	65	11.5
Respiration	20	9.2
Photosaturation and photoinhibition	40	5.5

[a] Taken from Leite and Hallenbeck, (2012).

due to their realtively easy maintenance, low cost and high surface to volume ratio (Suali and Sarbatly, 2012). Among the advantages of using photobioreactors are resistance to contamination by wild algae strains or herbivores, high productivity per unit area, and the possibility of easily controlling various parameters (Table 2), including pH, temperature, and light intensity. The PBR can be placed indoors or outdoors, using sunlight, artificial light or a mixture of both. An interesting variation of a lighting system is the use of optical fibers to carry the outdoor sunlight into an indoor culture (Chen et al., 2008). Artificial light can be provided by any regular light source such as tungsten or fluorescent bulbs. The use of LEDs (light emitting diodes) is increasing due to their low heat generation, lower power consumption and the specificity of the wavelength of emitted light, allowing the restriction of light to PAR (photosynthetic active radiation) and even the study of the influence of different wavelengths and intensities on these microorganisms. A recent study showed that different wavelengths may have a significant influence on biomass and lipid productivity, as well as on the lipid profile. A locally isolated strain of *Nannochloropsis* showed a higher growth rate, lipid productivity and different lipid profile under blue light (470 nm) when compared with growth under white, red (680 nm) or green (550 nm) (Das et al., 2011b).

4.2. Open ponds

Open pond cultivation is carried out in shallow basins open to the environment. The most common types are raceway, circular, inclined and unmixed. They are considered relatively inexpensive and easy to construct, as long as the area is relatively flat. Cultivation can be made directly over the soil or some simple surface covering can be used to minimize water loss due to seepage, and other improvements can be made to increase solar energy capture, and decrease contamination issues. Mixing can be provided effectively with low cost and low energy consuming paddle wheels, which can be enough to maintain aeration and nutrient dispersion. Due to the low depth and large surface area, water loss through evaporation can become a major issue, limiting its operation to areas where low cost water is available. Marine waters and wastewaters are good matches for this system, as environmental and sustainability issues would prevent large open pond cultivation using potable water.

Operation and maintenance costs are relatively low. Thus, this system is capable of generating biomass production at the best price. There is already some experience on large scale production using these types of systems, either in pilot projects partially funded by the government, in wastewater treatment plants, where it is used in secondary or tertiary treatment of sewage, or in commercial scale algal cultivation for the health food market. As a bioproduction system, its simplicity is a double-edged sword. The contamination risk level is high and a strain with high lipid productivity can easily be overrun by a fast growing wild strain (Sheehan et al., 2003). Another dangerous type of contamination is herbivores. There is not much information available on how to deal with predation, but it is well known that they are capable of clearing a high density pond in a matter of days.

4.3. Productivity

The purpose of the mass algal culture and local weather conditions may make the choice of system obvious. However, excluding these special needs and conditions, the main comparison between the two systems is principally cost and productivity. Regarding the productivity per unit area or volume, PBR are said to outperform open ponds. PBR structures can be made vertically, creating a high density cell culture in three dimensions. Open ponds require larger and more level cultivation areas to achieve the same productivity. The low mixing rate of open ponds intensifies the self-shading effect due to cell concentration (Table 3), and the physical structure of open ponds prevents proper aeration, causing a low medium CO_2 partial pressure. These effects limit the productivity rate per unit of area and volume, requiring a larger area to achieve the productivity of a PBR.

Of course PBRs have several advantages over open ponds as a cultivation system. However, an open pond is considerably cheaper. The total cost can be analyzed as infrastructure costs (CapEx), maintenance costs and operational costs (OpEx). All are in favor of open ponds. The installation and maintenance costs of PBRs may prove prohibitive for the production of low cost compounds, but acceptable for the nutraceutical industry. Carotenoids and some poly-unsaturated fatty acids (PUFAs), such as omega-3 and linoleic acid, are some of the high value products that can be produced in a microalgal system, where closed cultivation is more easily justified. Nevertheless, the development of PBRs is being pushed by research on microalgal biodiesel. Different PBR designs are being tested and some studies showed high productivities using systems requiring only relatively simple operation and maintenance. Using innovative 110L flat green wall photobioreactors, a production of 204 mg L^{-1} d^{-1} was reached (Rodolfi et al., 2009). Thus, open ponds offer a cheaper operation, but at the expense of productivity. Long term studies with outdoor open ponds have reported productivities ranging from 20 to 50 mg L^{-1} day^{-1} (Das et al., 2011a; Moazami et al., 2012).

4.4. CO_2 enrichment

One approach to raising productivity is to increase the concentration of CO_2 (Sheehan et al., 2003; Lin et al., 2012). In fact, the enzyme responsible for CO_2 fixation, Rubisco (Ribulose-1,5-bisphosphate carboxylase oxygenase), has a low affinity for CO_2 and also functions as an oxidase of 1,5-bisphosphate, interacting with O_2. Therefore, O_2 is a competitive inhibitor with CO_2 and since to the atmospheric concentration of CO_2 is much lower than that of O_2, oxygen can have a significant effect. Evolutionarily this problem has been managed by the development of carbon concentration mechanisms (CCM), where the cell locally increases the CO_2 concentration around the Rubisco enzyme to ensure its function in CO_2 fixation (Giordano et al., 2005). This mechanism is wide spread amongst the algae and illustrates the advantages of raising the CO_2 concentration in mass cultures. Indeed, sparging CO_2 into the culture medium is known to increase its cellular concentration and two different approaches are frequently reported, the use of CO_2 to adjust the pH, and CO_2 enrichment as a way to mitigate flue gases (Grobbelaar, 2000; Rodolfi et al., 2009; Yoo et al., 2010; McGinn et al., 2011). Of course any feedstock used in large scale production will play an important role on the final price and CO_2 is not an exception. Thus, this type production should optimally be coupled to a bioremediation process.

5. From biomass to biodiesel

5.1. Harvesting

In a general sense, the production of microalgal biodiesel is very similar to the production of first generation biodiesel. The biomass is produced, harvested; lipids are extracted and then processed through transesterification into FAMES (Fatty Acid Methyl Ester), commonly called biodiesel. However, unlike oil seed plants, harvesting microalgal cells can prove to be quite challenging. The tiny cells floating in water cannot be accessed as easily as macroscopic plants, and consequently oil extraction gets more complicated than the centuries old press procedure traditionally used for oil seeds. Moreover, algal cultures are very dilute, usually around 1% for autotrophic growth up to 10% for heterotrophic growth (Wu and Shi, 2007; Gouveia and Oliveira, 2008), and dewatering is necessary prior to biomass use. Many standard techniques have been evaluated for use in mass algal cultivation and their limitations are reviewed in detail elsewhere (Molina Grima et al., 2003; Mata et al., 2010; Zhu and Ketola, 2012).

Thus, harvesting can be done at once or divided into different steps, each one varying depending upon the desired final total solids concentration. Usually, the first step produces nothing more than a green slurry, and further drying may be necessary. Of course, the choice of harvest method will vary depending on the ultimate use of the biomass. Nutraceutical products may require physical processes for harvesting, thus avoiding chemical contamination, and maintaining the product's natural characteristics. In this case, the high value of the product will compensate for the high cost and energy intensity of the method. Continuous centrifugation is the preferred method when the algal culture will be used for fish feeding purposes, due to a longer shelf-life. This method is very effective and it is still the most widely used due to its efficiency and well documented techniques (Heasman et al., 2000; Molina Grima et al., 2003). However, it is among the low value high demand products, such as biodiesel, that harvesting and dewatering methodologies play a key role and, the use of energy intensive process for harvesting, such as centrifugation and tangential filtration, can represent 20–57% of the final biomass cost (Molina Grima et al., 2003; Van Den Hende et al., 2011) and compromise the overall net energy ratio (Sander and Murthy, 2010).

5.1.1. Possible promising harvesting technologies

Thus, one major hurdle in developing a viable biodiesel from microalgae production process is how to effectively harvest the biomass in a cost-effective manner (Uduman et al., 2010). A variety of methods are potentially available, including; centrifugation, flocculation, filtration, sedimentation, and mat formation, and, as reviewed below, a number of recent studies provide some hope for the near-term development of a cost-effective harvesting technology. Of course, how effective many of these are can sometimes be species dependent. Thus, acceptable harvesting procedures can be highly dependent on the cultivation method. Although, as discussed above, open pond systems are to be preferred for biofuels production for a number of reasons, these are likely to produce mixed cultures, or at the very least, monocultures whose composition differs according to location specific conditions. Thus, techniques that rely on species specific characteristics can probably only be successfully used with cultures grown on photobioreactors where, at least in principle, some sort of species control is possible.

5.1.2. Centrifugation

As noted above, centrifugation has been the method of choice in small scale studies since it is highly effective and capable of harvesting all but the most fragile species. Yet, it has been argued that this method is too energy intensive for application to what is essentially a low value product where there is a need to keep as high a NER (Net Energy Ratio) as possible. This is undoubtedly true if high levels of removal are sought. However, it has recently been argued that acceptable costs can be obtained by increasing the flow (i.e. volumetric throughput) and accepting a lower capture efficiency (Dassey and Theegala, 2013). These authors found that energy consumption could be decreased by 82% when only 28% of the algal biomass was collected resulting in a harvesting cost that they estimated to be \$0.864/L oil.

5.1.3. Flocculation

Flocculation is a well-known process that has been used for years to remove algae and other suspended particles from water during treatment to produce potable water. In this process externally added compound causes the suspended algae to form flocs, which if of the correct size, will freely sediment. In fact, floc formation is a physic-chemical process and the resulting particle size is a function of mixing speed (Hallenbeck, 1943). Due to the negative charge of microalgal cell walls, they tend remain dispersed in solution. Flocculation agents can neutralize this charge, causing the cells to aggregate and settle, which facilitates the harvest process. Chemical flocculation methods and agents that can be used in microalgal cultures have been systematically investigated (Molina Grima et al., 2003; Uduman et al., 2010; Beach et al., 2012; Riaño et al., 2012). A desirable flocculant should be non-toxic, recyclable, inexpensive, and efficient at low concentrations. Due to the massive scale predicted for production of biodiesel, any chemical needed for the biomass cultivation or processing will have a significant impact on market price. Thus, recycling the compounds used for algal cultivation and processing is both an economic and a sustainability issue.

Various chemical flocculants can be applied, alum (hydrated aluminum potassium sulfate) or alkali are traditionally used, but cannot be considered for application in harvesting microalgae for biofuels production because, in addition to cost considerations, their toxic nature precludes further use of the algal biomass, for example for animal feed, after lipid extraction. However, this process might be adapted to make a cost-effective harvesting technology for biofuels production from microalgae if the right compound could be found. Moreover, a recent study suggests that previously projected costs might be too high as it was found that the amount of flocculant required varied with the logarithm of cell density instead of linearly. One widely accepted theory of flocculation is that it works through charge neutralization; the compound added (an alkali normally), neutralizes the negative charges on the surface of the algal cell thus allowing aggregation. Thus, this theory might be thought to predict a requirement for flocculant that increases linearly with cell number. Contrary to this, highly dense cultures were found to require substantially less flocculant, thus potentially substantially reducing costs (Schlesinger et al., 2012). That study proposed that cost effective flocculation using a mixture of calcium and magnesium hydroxides, with a cost of less than \$10.00 per ton of algal biomass, could be achieved, due to the low concentration of flocculating agents required (<12 μM) and the high density of the cell culture used (6×10^7 cell/ml). However, flocculant demand will probably also be a function of the particular algal species since coagulation properties are dependent upon a complex set of characteristics including cell size and extracellular polysaccharide production (Eldridge et al., 2012). In an interesting recent development, it has been shown up to 99% of the biomass can be effectively recovered using ammonia (Chen et al., 2012), which can be recycled into the culture as a source of nitrogen after neutralization of the pH. It is not known if this procedure can be applied to a wide variety of alga species.

Bio-flocculation is a promising and poorly explored alternative. Some algal strains have a natural ability to auto-flocculate under some specific conditions (Olguín, 2012), while others can be flocculated by the addition of a bacterial culture (Kim et al., 2011). This suggests that novel compounds might be found that could be used as flocculants and that would avoid at least some of the disadvantages of presently used chemical flocculants. One example is the newly described flocculant excreted by cultures of *Solibacillus silvestris* which has been shown to efficiently flocculate cultures of the marine microalgae *Nannochloropsis oceanica* and which can be reused (Wan et al., 2012). Likewise, a bioflocculant has been isolated from an autofloculating *Scenedesmus* (Guo et al., 2013). Of course, it is desirable that any flocculant be of use with a wide variety of species.

5.1.4. Filtration

Filtration can be a very effective method of harvest if the species is large enough or grows in filaments. However, again this implies that the desired species be maintained as a nearly homogenous monoculture. Most microalgae are too small to be effectively harvested this way since their small size and extracellular material quickly clog filters that have been tested.

5.1.5. Sedimentation/flotation

Some microalgal species have the peculiar properties of either sedimenting or floating in the absence of sufficient mixing. While this property could be used to advantage in a least an initial dewatering process, once again the applicability of this method would require a high level of species control during cultivation. Moreover, these properties, while possibly leading to low cost harvesting, may also negatively impact mixing requirements since it may be more difficult to maintain these strains as evenly dispersed cells during cultivation.

5.1.6. Biofilm formation

Species that readily form biofilms have been little studied for biofuels production since it is obviously difficult to maintain them as a homogenous suspension in the cultivation medium. However, several recent studies, with two different systems, have shown that this kind of growth mode can offer the ease of simple mechanical harvesting, leading to slurries with a dry weight content of 9–16%. In one case, algae were grown on a rotating drum in what was otherwise an open pond system, and simple mechanical harvesting was achieved by simply unspooling and scraping the cotton "rope" fiber that was used (Christenson and Sims, 2012). In another approach, the algae were grown on a flat surface which was drip-watered. At the end of the growth period the algae were recovered by simple mechanical scraping (Ozkan et al., 2012). Not only was harvesting greatly simplified in both cases, both protocols achieved high rates of biomass production at respectable light conversion efficiencies.

5.2. Lipid extraction and transesterification

The lipids produced by microalgae are usually between 12 and 22 carbons long and can be saturated or unsaturated (Medina et al., 1998). These can be directed for membrane synthesis (polar lipids) or stored as carbon reserve (neutral lipids). For biodiesel production, saturated fatty acids between 12 and 16 carbons are desirable (Srivastava and Prasad, 2012), with the ideal proportion varying depending upon the local climate (Dunn and Bagby, 1995).

In line with the efforts to find a solution for low cost harvesting of microalgal biomass, a great deal of research on lipid extraction is examining wet extraction methods since the harvest process can be simpler and cheaper if biomass with a very low water content is not required. Direct or wet transesterification, is simply the omission of the extraction step, using the whole biomass as feedstock for the reaction. Surprisingly, the exclusion of the extraction step was found to raise efficiency, increasing the lipid yield per gram of biomass (Griffiths et al., 2010). The major drawbacks of this method are the variation of efficiency when applied to different strains, and the use of volatile solvents which are dangerous pollutants.

A more ecological option would be extraction using switchable solvents These solvents can be either a polar or a non-polar, and can be switched between the two by bubbling with N_2 or CO_2 respectively (Jessop et al., 2005). In a polar configuration, they are highly miscible with water, facilitating entry into the cell and contact with the neutral lipids. Once switched back to a non-polar state, they will extract the lipids out of the cells and out of the aqueous phase. Recovery of the lipids and solvent can be performed by switching to polar and then back to nonpolar, avoiding the distillation process commonly used for volatile solvents, and increasing the recovery rate. This green chemistry has been tested with vegetable oil (Phan et al., 2009), yeast, and microalgal biomass (Young et al., 2010; Boyd et al., 2012). Lipid extraction of unconcentrated algal cultures itself might be feasible (Samorì et al., 2010).

When just a simple extraction is used, the fraction, primarily TAGs, must be transesterified to produce molecules, acyl-esters of the free fatty acids. This involves the substitution of an alcohol for the glycerol found in the TAG, with either methanol or ethanol being used, giving FAMEs (fatty acid methyl esters) or FAEEs (fatty acid ethyl esters). This reaction requires a catalyst, either an acid or a base, to occur at reasonable rates at relatively low temperatures and pressures. In practice, the same compounds, methanol (which is cheaper than ethanol but produced from fossil fuel), and sodium (or potassium) hydroxide or sodium methoxide, the same reagents used in production of biodiesel from oil seeds, are commonly used.

6. Biotechnology of microalgal biofuels

Throughout this review various issues that apply to the biotechnology of microalgal biofuels have been discussed. Here we specifically highlight some specific biotechnology issues that are important in the development of large scale algal biofuels production.

6.1. Water and nutrient supply

At large scale the demands for water, for makeup for evaporative losses (especially relevant to open ponds in arid areas), and for nutrients, are enormous. If indeed production is to be done in a fashion that does not compete with food production, and in terms of cost-effectiveness, cheap sources of the major nutrients require, especially the major ones, nitrogen and phosphorous, must be used. In fact, both conditions can be met by using suitable wastewater. Of course, different wastewater streams vary widely in their composition and nutrient removal (uptake) appears to be a complex function of a number of factors including nutrient levels and species (Cai et al., 2013). The use of algae for nutrient removal from municipal wastewater has been extensively investigated and in general this nutrient stream provides a good microalgal growth medium. Other wastestreams promise to also provide most of the nutrients for abundant microalgal growth (Cabanelas et al., 2013; Cho et al., 2013). Coupling biofuels production with wastewater treatment makes sense since it results in considerable energy savings, important in improving the NER of an algal production process (Beal et al., 2012).

6.2. Strain selection, cultivation and harvesting

The various cultivation strategies were discussed above for the general case of microalgal biomass production. With existing technology it is obvious that for a low value product such as a biofuel open ponds must be used, photobioreactors are simply too expensive, as discussed above (Section 4). A simple calculation based on possible solar energy inputs and maximum photosynthetic conversion efficiencies shows that the resulting energy value per square meter allows very little capital expenditure for cultivation facilities. Therefore, although specific laboratory strains are attractive since they have been shown to produce high levels of lipids, i.e. *Botryococcos braunii*, they cannot be used in practice in an open system since they will quickly be overrun by indigenous species. One solution is to isolate species native to the particular locale, more likely to be able to compete and prosper under the prevailing climatic conditions if provided in a healthy enough inoculum. Cost-effective harvesting is of course a major unsolved challenge, and several new advances have already been discussed above (Section 5.1.1).

6.3. Oil extraction and transesterification

A variety of different novel extraction and conversion procedures are under active investigation with the goal of obtaining high biodiesel yields in an energy efficient manner that doesn't require extensive use of toxic solvents. As pointed out above, the use of organic solvents, the traditional method for oil extraction from oil seeds, should be avoided both from the perspective of eliminating possible toxic pollutants, but also from an energetic point of view given the energy intensive processes required for solvent recovery. One elegant way around this impasse is the use of switchable solvents (Boyd et al., 2012). Since they can be interconverted between having a polar and a nonpolar character simply by using CO_2, solvent recovery through distillation is not required. Moreover, relatively environmentally benign solvents can be used.

A promising recent development is the demonstration of a wet lipid extraction procedure (Sathish and Sims, 2012). In this procedure the harvested algae do not require complete drying prior to extraction, close to 80% of the lipids susceptible to transesterification could be recovered from wet algal biomass (84% moisture content). Other technologies aim at increasing extraction yields through some form of cell disruption, facilitating solvent access. Pulsed field electroporation seems particularly promising in this regard due to its relatively low energy demand (de Boer et al., 2012).

6.4. Economic analysis

Of course, before moving to very large scale microalgal biofuels production, the production system needs to be subjected to a detailed LCA (life cycle assessment), to determine possible environmental impacts, a determination of NER (net energy ratio), and an economic analysis. However, to do this in a meaningful way requires specific inputs on system components, and since many of the outstanding questions raised here; cultivation method (open ponds versus photobioreactors), harvesting technologies, and even extraction and transesterification reactions, remain to be answered, this cannot really be done in a meaningful way at present.

Moreover, an economic analysis which compares the price at the pump of a biofuel with that of a fossil fuel is in fact wrong. For biofuels a metaeconomic analysis is necessary that takes into account indirect costs associated with fossil fuel production and use. A quick overview suggests that there are in fact many hidden costs to fossil fuel use and that the "real" cost of gasoline or diesel is significantly higher than the price paid by the consumer at the pump. The additional costs of course must be paid either now or later in other ways, typically through a higher tax burden. There are of course direct subsidies to the fossil fuel industry, estimated at about $50 billion (USD) over the next ten years in the US alone (EESI, 2011).

Although this number is significant, the real hidden costs of fossil fuel use are much higher. Damages from external effects, such as impacts on the health system, but not including those related to climate change, ecosystems, infrastructure and security were estimated at $120 billion for the US in 2005 alone (National Research Council, 2010). To this of course must be added the costs of climate change due to fossil fuel use. One way to estimate the damage is to look at the cost of adapting to climate change, although this does not provide the actual full costs incurred since this represents less than full mitigation. An initial international study estimated these costs at $49 to 171 billion (USD) per year (UNFCCC, 2007) and it has been argued that this is in fact an underestimate (Parry et al., 2009). Of course, these estimates are highly dependent on the accumulated of atmospheric CO_2 burden over time as well as a great deal of uncertainty as to actual impacts. Thus, determining what the competitive cost of a biofuel really should be will require detailed economic analysis. In addition, as mentioned above, detailed costing is not possible given the many uncertainties in the design specifics of a practical algal biodiesel plant. Thus, a realistic cost analysis is impossible at present.

7. Conclusion

The production of biofuels using microalgae is promising since of all photosynthetic organisms they have the highest growth rates, and they can be cultivated using non-arable land with wastewater as a source of nutrients. However, much research is still needed before the practical production of biofuels from microalgae can become a reality due to uncertainties as to cultivation strategies, the lack of effective low cost harvesting methodologies, and the need for an oil extraction and biodiesel conversion technology adapted to algal biomass. However, recent advances in some of these areas are encouraging, and the next decade will probably see the successful demonstration of algal cultivation for biodiesel production on the pilot scale or larger.

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