

Microalgae: the next best alternative to fossil fuels after biomass. A review

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Abstract

It is expected that 84% of the global energy demands will be met through fossil fuels in 2030 due to increasing energy needs. However, due to their impact on the environment through the emission of anthropogenic green house gases, biofuels were introduced as alternative sources of energy. Biofuels of plant origin for the transport sector proved to be controversial due to competition for food production, fertile land and expensive production processes. As a secondary alternative, microalgae such as *Scenedesmus obliquus*, *Chlorella vulgaris* and *Nannochloropsis* sp. were found to be suitable candidates for liquid biofuels production. This review describes the production of transportation liquid biofuels from plant biomass and microalgae. Information is provided on how the controversies related to plant biomass lead to the use of algal biomass. The production processes involved in both generations are discussed and highlighted. Furthermore, details on the production of secondary products such as pigments, feed additives and valuable secondary metabolites are also provided.

Introduction

Energy is one of the essential elements that is needed to sustain human survival and development. Since the mid-18th century, the invention of new technologies requiring high energy density input caused a sudden increase in the use of fossil fuels such as coal.¹ Since then, the demand in energy in the form of fossil fuels has not dropped.² It is known that these non-renewable sources of energy will be depleted but with the current state of oil reserves and based on the global demand, it is expected that crude oil supply will be adequate through 2050.^{3,4}

The carbon dioxide generated from the use of such fuels has major negative impacts on the environment such as increased temperature resulting in water scarcity in dry regions, torrential rainfall in

wet regions, melting glaciers increasing sea levels and stronger and more frequent heat waves.⁵⁻⁷ These are being caused by the increasing global temperature through the past 3 decades.⁸ With so many issues impacting the planet, it is a must to find reliable alternative sources of energy having long term positive effect such as biofuels.

In its early development through the 20th century, the use of plant biomass was found to hold great potential in the form of biofuels.⁹ Bioethanol production from agricultural sources has demonstrated a lot of potential as a source of fuel with a prime example of Brazil blending ethanol, produced from sugarcane, to all of its gasoline to yield a 24% blend.¹⁰ Other non-food crops such as *Arundo donax* and *Pongamia pinnata* were sought but do not alleviate the issue of competition for food production.¹¹ Moreover, it requires 1540 Mha of land to produce 172 L/ha of oil from corn which is in direct conflict with agricultural practices for food for consumption.¹²

Due to greenhouse gas (GHG) emissions and food production issues, other environmental friendly sources such as microalgae were bound to be researched as the 3rd generation biofuels.¹² They are photosynthetic microorganisms synthesizing lipids and carbohydrates that can be used for biofuel production.^{13,14} Microalgae culture is considered as a carbon neutral process and does not compete with food crop production.^{15,16} In comparison to terrestrial crops, microalgae fuels have a higher yield of 30-100 times more energy per hectare of land used which is possible with the use of microalgae such as *Porphyridium cruentum* and *Scenedesmus dimorphus* which can reach 40-57% and 21-52% carbohydrates on a dry matter basis, respectively.¹⁷ With respect to lipids, *Nannochloropsis salina* was found to reach 29% lipid content when cultured with wastewater supply.¹⁸

This review offers information on how the overuse of fossil fuels lead to the application of plant biomass firstly and then microalgae for biofuels production. The various sources of plant biomass and the major processes involved in the production chain are introduced along with the impacts of their use. For the algal biomass section, the currently used microalgae strains, culture conditions and biomass processing techniques are extensively developed with additional information on the other uses of microalgae.

Fossil fuels

Primary source of energy

Due to the unavailability of the conditions that existed millions of years ago that

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transformed organic material into fossil fuels, it was made evident that the latter are bound to be completely exhausted as they are non renewable.¹⁹ It has been envisaged that in the next 30 years, petroleum will no longer be available.³ However coal, oil and natural gas are currently the major sources of energy satisfying the global demand.²⁰ It is estimated that the demand in oil will increase to 103.8 mb/d by 2022 from an initial average of 96.6 mb/d in 2016.⁴ Moreover, it is expected that fossil fuels will continue to meet up to 84% of the energy demand in 2030.²¹

Demands in oil and gasoline have been increasing for decades in various countries. In the case of Brazil, gasoline consumption grew at a rate of 7.5% from 1990 to 1999.²² Similar trends were observed in countries like Lebanon and China in the past decade.²³ These surges in fuel demands can be accounted to the growth of car imports and the growth in the population requiring more energy such as electricity in countries like Brazil and Senegal.^{22,24} All of such demands are met through global supplies but many producing countries such as Iran have gone beyond their fuel production capacities due to high levels of subsidies and very low prices of fuels.²⁵ This results in a very high local use and demand of the fuels.

Impact on health and environment

The utilization of fossil fuels has been subject to various controversies following the discovery of the global warming effect and GHG emissions.²⁶ CO₂ dominates the entirety of the GHGs by 90%.²⁷ The two major sectors impacting the CO₂ emissions are electricity & heat (42%) and transport (24%).^{27,28} In 2015, the global CO₂ emissions reached 32.3 GtCO₂ compared to 32.2 GtCO₂ in 2013. Although the increase seems very small, the consequences of the emissions are still having a destructive influence on society and the environment. In 2012, there were 3.7 million deaths due to ambient air pollution on the planet.²⁹ Impacts on the environment were identified as follows; dry regions experiencing water scarcity due to increased temperature, torrential rainfall occurring in wet regions, and more frequent heat waves.⁵⁻⁷ Sea levels are increasing due to melting glaciers with sea height variations of 21.6 (± 0.80)mm in February 2000 rising to 89.9 (±0.80)mm in October 2018.³⁰ These are being caused by the increasing global temperature through the past 3 decades.⁸

Another factor contributing to high CO₂ emissions is subsidiary. The high levels of fuel subsidiaries in countries like Iran and Saudi Arabia, which are part of the Middle East and North Africa and which are considered as the largest contributors to CO₂ emissions, renders the current situation of global warming difficult to overcome.³¹ This drives the aspect of efficient energy use and production of energy efficient goods out of context and the same situation applies to renewable energy industries.³²

The Kyoto Protocol in 1998 and the 2015 Paris Climate Conference were initiated to implement and elaborate policies and measures to reduce CO₂ emissions and keeping global warming below 2°C.^{33,34} For the transport sector, a lot of research is being done to reduce CO₂ emissions in the long term through the enforcement of poli-

cies and counter measures.^{35,36} However, due to their slow impact,³⁷ the most suitable method is to attack the current issue by modifying the fuel in use itself through the production of biodiesel and bioethanol.^{9,38} When the conflicts between increasing fuel demands and environmental issues were identified, 2 strategies were evaluated; modifying engines according to the fuel or modifying the fuel to the engine. However, it proved to be too costly to produce such engines and their selling prices were very high due to low production numbers.⁹ Therefore, the use of bioethanol in gasoline and biodiesel in diesel remain the cheaper alternative.³⁹

Biofuels: 1st and 2nd generations

Production status

In the meantime, research was already ongoing with the objective to seek alternative sources of fuels. Biofuels such as bioethanol and biodiesel were found to be among the most promising and attractive solutions to the replacement of fossil fuels in the transportation sector.⁴⁰ First generation biofuels are primarily derived from crop plant products such as sugar and starch for ethanol while vegetable oils and animal fats for biodiesel production.^{41,42} The major plants investigated and utilized were sugar cane, corn and wheat for ethanol while palm oil, sunflower seeds, castor seeds, rapeseed and soybean for biodiesel among many others.⁴³⁻⁴⁵

In 2016, the global biofuel production reached a rate of 2.35 mb/d and represented 4% in terms of world road transport fuel. Ethanol production in 2016 was at 1.73 mb/d while that of biodiesel was recorded at 620 kb/d. With annual growths of 2.5% and 4% for ethanol and biodiesel, it is expected that the global output by 2022 will reach the 2 mb/d and 800 kb/d respectively.⁴ Forecasts (Table 1) on the global production

are crucial in the advancement of biofuels as a mean against global warming.

In the US, the average retail prices of biofuel blends E85, B20 and B99/B100 recorded on first January 2018 were \$2.68, \$2.55 and \$3.41 per gallon respectively. However, when compared to the gasoline (\$2.50/gallon) and diesel (\$2.63/gallon), the prices of biofuel blends are higher.⁴⁶ The prevailing trend is that the higher the blend used, the higher the price. This situation is due to the fact that liquid biofuels provide less energy density in comparison to fossil fuels when measured in equivalent units. Therefore, more fuel is needed to cover distances and thus creates more demands in ethanol and diesel. Additional contributing factors are lack of financial support and international trade barriers.⁴⁷ Financial support as subsidiaries has greatly helped Thailand in 2008 where E85 was 30-40% cheaper than gasoline.⁴⁸ However, this scenario cannot be resolved with the use of food crops for biofuels as it is a highly debatable topic, which has a major impact on the price on biofuels. As it is, availability of the feedstock on the market according to seasonal patterns is very important as it dictates the price of the resulting biofuel.⁴⁹

Agricultural point of view: plants for food or fuels?

The world population is currently at 7.6 billion and is increasing.² With so many individuals to feed, it is imperative that agricultural productivity be increased by 70% to meet demands for food in 2050.⁵⁰ Developing countries are faced with social and economic issues on the use of food crops for fuel or for consumption.^{51,52} Producing biofuels using food commodities decreases the availability of these commodities on food markets. This results in a chain reaction where the prices of these products increase and thus causes more land to be brought into production leading to an increase in biodiversity loss.^{53,54}

Table 1. The global ethanol and biodiesel productions recorded in 2016 from major countries and predictions for 2022.⁴

Biofuel	Country	Major feedstock(s)	Production for 2016 (kb/d)	Forecast for 2022 (kb/d)
Ethanol	United States	Corn	990	1015
	Brazil	Sugarcane	470	620
	China	Corn	50	65
	OECD Europe	Corn, wheat, sugar beet	80	115
	India	Molasses	19	36
	Thailand	Molasses	21	40
	United States	Soybean	98	125
	Brazil	Soybean	66	90
Biodiesel	OECD Europe	Hydrogenated vegetable oil	227	270
	Indonesia	Palm oil	50	110
	Malaysia	Palm oil	15	24
	Argentina	Soybean	50	60

Improving biofuel output per area of land can be considered only as a small fragment of a solution for increasing energy efficiency per unit area of biomass.⁵⁵ However, food provision and bioenergy cannot be treated in isolation from one another. To address this situation, the application of Integrated Food Energy Systems (IFES) where liquid biofuels, food and energy are all simultaneously produced seems promising.⁵⁶ The concept of intercropping various species of crop plants such as maize, pigeon peas and sorghums is a potential way of assuring food production, land use and residual biomass conversion to biofuels.⁵⁶ However, it is still a risky business when there are natural disasters. Between 2003 and 2013, 78 natural disasters occurred across the globe causing a total of \$30 billion of damages to agriculture.⁵⁷ Moreover, there are 925 million people that are undernourished and thus the focus with food production is driven to food security concerns.⁵⁸

The 2nd generation biofuels

Lignocellulosic biomass for ethanol

Biofuel production from starch-rich materials and food crops proved to be inefficient for large scale biofuel production due to the increase in food commodity prices, competition for food and land for crop plantation.⁵³ However, non-food crops and plant-based materials were found to be potential candidates in the form of lignocellulosic materials such as wood, paper, agricultural residues and specific energy crops.⁵⁹ These materials are easily available, renewable and inexpensive for the production of bioethanol.^{60,61} They consist mainly of cellulose and hemicellulose which are the sources of carbohydrates.^{62,63}

As mentioned earlier, lignocellulosic biomass can be from agricultural residues as well as non agricultural products. Non agricultural products and plant-based materials include newspapers, hardwood and softwood among many others.⁶⁴ Agricultural residues consist of sugarcane bagasse, corn cobs, corn stover and other non crop-plants such as spruce, *Arundo donax* and switch grass.^{60,65-69} Lignocellulosic ethanol production starts with biomass pretreatment, hydrolysis, fermentation, distillation and drying.⁷⁰ Improvements to the processes lead to a more complex production system.^{60,71,72} Following pretreatment, the biomass undergoes two further steps, hydrolysis and fermentation (Figure 1). The implementation of the technology on industrial scale is yet unknown.⁷³ However, even though lignocellulosic biomass has proved to hold enough potential for ethanol production and that it does not compete directly

with food production, it still does require land for cultivation. This triggers once again the debate on land use for food production over fuel production.

Non-edible crops for biodiesel

The first generation of biodiesel came mainly from edible vegetable oils of soybeans, palm oil, rapeseed, peanut and coconut. Other sources include the non-edible vegetable oil (*Jatropha curcas*, *Croton megalocarpus*, *Pongamia pinnata*), waste or recycled oils and animal fats.⁷⁴⁻⁷⁷ The world biodiesel production is generated from edible oils at more than 95% (rapeseed at 84% and sunflower oil at 13%) with increasing production values over the years (Table 2).⁷⁸ The US cultivates soybean extensively as a biodiesel feed stock as well as food source but can only meet 6% of the energy demands.⁷⁹

The production of biodiesel depends on the methods used, including transesterification, thermal cracking and micro-emulsions.^{81,82} The basic process in biodiesel production is the transesterification step (Figure 2) where fatty acids (triglycerides) are converted into methyl esters (biodiesel). Nowadays, with engine developments and improved biodiesel processing, the US manufactures B100, B20, B5 and B2 biodiesel blends for accentuated efficiency

and combustion.⁸³ Combinations of oils of various origins are also evaluated for their performance as fuels.

Microalgae: the 3rd generation biofuel

Better than plants

With all of the mentioned issues on the use of crops for biofuels production, the next promising resource was found to be microalgae. Microalgae have been receiving a lot of attention due to their characteristics in terms of high lipid and carbohydrate contents and applications.⁸⁴ These photoautotrophs have existed for billions of years capturing CO₂ and thus provided suitable conditions for life to exist and evolve.¹⁷ Even though microalgae thrive on land and in aquatic environments, there are only 30,000 species that are currently known.⁸⁵ They exist as both prokaryotes and eukaryotes consisting of a total of 11 divisions of which the groups *Cyanophyceae* (blue-green algae), *Chrysophyceae* (golden algae) and *Chlorophyceae* (green algae) are the mostly exploited ones.¹³ With the issues of second generation biofuels production such as land use, costly pretreatments for lignin removal

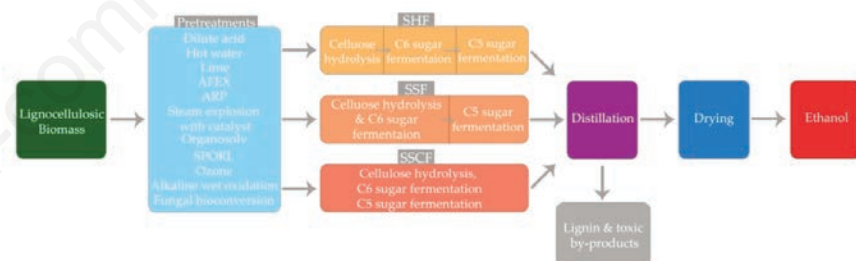


Figure 1. Flow diagram illustrating the different pretreatments and pathways involved in bioethanol production from lignocellulosic biomass.^{70,74} SHF: separate hydrolysis and fermentation; SSF: simultaneous saccharification and fermentation; SSCF: simultaneous saccharification and co-fermentation.

Table 2. Annual global production values of some biofuel feed-stocks.⁸⁰

Feedstock	Production per year (Mt)		
	2000	2010	2016
Soybean	161.3	264.9	334.9
Castor oil	1.4	1.7	1.8
Coconut	51.2	60.1	59.0
Palm oil	22.2	45.8	0.0
Rapeseed	39.6	59.9	68.9
Safflower	0.6	0.7	0.9

and food crop productions, microalgae offer a more convenient approach to alleviating the supply of biofuels as they do not possess lignin.⁸⁶

Just like plants, these organisms can produce carbohydrates, lipids and proteins as well as other interesting compounds such as astaxanthin and β -carotene.⁸⁷ The cellular contents of microalgae vary from species to species (Table 3). Microalgae can synthesize between 30-70% of oil (by weight) in biomass. This particular characteristic makes these microalgae suitable candidates for biodiesel production. In comparison to production values, corn requires 1540 Mha of land to produce 172 L/ha of oil while microalgae would need 2 Mha of land area to yield 136900 L/ha of biodiesel.¹²

Similar trends can be observed with bioethanol production as joint productions of both biofuels have been successful with microalgae.⁸⁸⁻⁹⁴ *Scenedesmus abundans* was investigated as a potential candidate for bioethanol production reaching a productivity of 0.103g of bioethanol per gram of dry weight algae.⁹⁵ It is safe to say that microalgae are the best potential sources of biofuels at the moment.

Nutrition and culture conditions

The concentrations of the various biochemical compounds within the microalgae are highly dependent on the environment. It is already known that changes in the environment such as light, temperature and nutrients affect the growth of microalgae and the accumulation of carbohydrates, proteins and lipids.⁹⁶ The basic parameters for microalgae culture consist of a light source, temperature between 16-27°C, pH ranging between 7-9 and salinity between 20-24g/L and mixing.¹³ Incapacity to provide optimal conditions can result in the culture collapsing or poor growth.

Effect of lighting parameters

Microalgae are photosynthetic organisms and thus require light to thrive. Typically, the light intensity used varies between 100 and 200 $\mu\text{E sec}^{-1}\text{m}^{-2}$ for productions depending on strains used.¹³ The effect of light source is limiting especially in terms of biomass production.⁹⁷ During culture, the higher the intensity (within permissible limits), the higher the penetration power of the light across the culture vessel and media. This was observed with *Dunaliella viridis* where irradiance was not powerful enough to allow cells to photosynthesize at a depth above 10cm.⁹⁸ The use of higher intensities can result in energy wastage and ultimately costs. This can be explained with respect to cells being unable to process light energy to biochemical ener-

gy. The excess energy is dissipated as heat to the environment.⁹⁹ Many attempts to modulate the effects of light on microalgae culture are ongoing. Trials aiming at optimizing the effect of photoperiod (light/darkness) are also ongoing. The most widely used settings are 12h:12h, 14h:10h or 24h:0h. It must be pointed out that photoperiods are essential for the production of ATP and NADPH during photosynthesis. Darkness allows the cells to produce biomolecules important for growth.¹⁰⁰

The growth rate is also dependent on temperature for optimal lipid production.¹⁰¹ Temperature is a very special parameter to observe as rises in the latter can be due to lighting, surrounding heating elements and other sources. Though normally ambient temperature is preferred for microalgae culture, it has been shown that this particular parameter may have inverse relationships with certain microalgae.¹⁰² The manipulation of these specific parameters have been reported as beneficial for certain metabolite production.¹⁰³

Effect of nitrogen and phosphorus

Microalgae prefer nutrient-enriched environments for growth and lack of nutrients can be detrimental as this is well observed in cyanobacteria. They tend to change structure and composition in communities in such situations.¹⁰⁴ Stress conditions on microalgae cultures of some species have also shown increased carbohydrates or lipids synthesis and vary from species to species. Aspects relating to stress conditions has been reviewed.¹⁰⁵ Microalgae such as *Chlorella vulgaris* and *Scenedesmus obliquus* were used as model organisms to evaluate the effect of nitrogen and phosphorus availability on biochemical composition and biomass yield. The effects showed that *S. obliquus* can accumulate more phosphorus (P) than *C. vulgaris* while the latter accumulates more nitrogen (N).¹⁰⁶ Nutrient starvation is a well known process which involves the accumulation of carbohydrates or lipids due to the metabolism of proteins.¹⁰⁷ However, some microalgae are not tolerant to such conditions and can

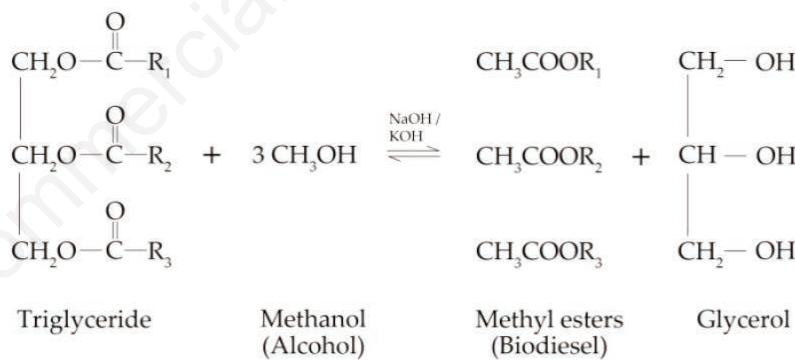


Figure 2. Transesterification reaction of oils to biodiesel.

Table 3. Biochemical characteristics of researched microalgae species (according to research parameters applied).

Microalgae species	Lipids	Proteins	Total Carbohydrates	Refs.
<i>Arthrospira platensis</i>	8.1	62.9	15.6	88
<i>Chlamydomonas reinhardtii</i>	21.0	48.0	17.0	17
<i>Chlorella</i> sp. MP-1	28.8	43.2	19.5	89
<i>Chlorella vulgaris</i>	2.0	57.0	26.0	90
<i>Chlorogloeopsis fritschii</i>	7.0	50.0	44.0	91
<i>Dunaliella salina</i>	9.1	5.4	69.7	92
<i>Dunaliella tertiolecta</i>	18.0-23.5	8.3-31.3	46.5-50.6	92
<i>Nannochloropsis</i> sp	8.1	16.7	-	93
<i>Porphyridium cruentum</i>	9.0-14.0	28.0-39.0	40.0-57.0	90
<i>Scenedesmus dimorphus</i>	16.0-40.0	8.0-18.0	21.0-52.0	17
<i>Tetraselmis maculata</i>	3.0	52.0	15.0	17

result in poor biomass productions. *Nannochloropsis* sp. was found to yield a low biomass upon N and P limitations as the interactive effects that these components have affect the lipid content.¹⁰⁸

Lipid accumulation can result for the conversion of the cellular-N pool (proteins) or by modifying their bio-synthetic pathways upon N deficiency in the media and excess of P. Therefore, hypothetically, for improved lipids productions, it is best to operate under batch conditions with excess P with *Nannochloropsis* sp. This scenario could improve biodiesel productions. Under similar stress conditions, carbohydrate content can also be amplified. This was observed with the microalgae *Chlorella vulgaris* where carbohydrate accumulation peaked at 22.4% compared to 16.0% under N-limitation.⁹⁶ Similar results were obtained with microalgae in the *Chrysothyceae* class, but the lack of N resulted in a low biomass even though the carbohydrate content was higher.⁹⁷ Microalgae are targeted for the production of biodiesel more than bioethanol due to their high lipid content.⁷⁶

Types of culture

Microalgae culture can be done in different ways such as photoautotrophic, heterotrophic and mixotrophic.¹⁰⁹ Photoautotrophy is the most commonly employed method for microalgae cultivations, and is present in the use of open pond and closed photobioreactor systems.¹¹⁰ This type of culture operates by using light as energy source, CO₂ from the surface air and presence of aerators in artificial ponds.¹¹¹ The microalgae grow by conducting photosynthesis where energy from the light source is stored as ATP and NADPH. They are then processed through the Calvin cycle to generate glucose as the energy source. This type of culture is the most economic one as it utilises sunlight as energy source. However, this criterion is also a limiting factor considering that photosynthesis will only occur in the presence of light. Artificial lightning is a means to counter this issue but is very costly to implement and run. It must be noted that microalgae culture is very diverse and culture systems must be carefully used depending on the desired end product.

Heterotrophy is different in the sense that an organic carbon source is present in the media and then absorbed and metabolized by the microalgae. The biomass yield is much higher and does not require light. The major carbon source used is normally glucose but can also include peptone and acetate.¹⁰⁹ The main disadvantages that this type of culture offers are the costs associat-

ed with the supply of the carbon source and potential contamination with bacteria/fungi. Growth of *Cyclotella cryptica* was recorded in heterotrophic conditions with glucose as carbon source. The cellular carbohydrate, protein and lipids contents were 360 mg/g, 260 mg/g and 165 mg/g respectively.¹⁰¹ A variation of heterotrophy is photoheterotrophy which is a culture condition where light is needed to activate Photosystem I while using sugar as the exclusive carbon source.¹⁰⁹ Each of these culture conditions has its advantages and disadvantages regarding the light and carbon sources, biomass yield and costs. However, depending on the goal of the culture, whether it is for biofuel production, feed production, or other applications, the condition will depend on the microalgae strain, investments and desired end product.

Mixotrophic systems occur where photosynthesis occur in the presence of organic compounds and CO₂.¹⁰⁹ In fact, it involves two stages: preliminary stage of heterotrophy due to high organic carbon content followed by photoautotrophy in the second stage.¹¹² Culture of *C. vulgaris* under mixotrophic conditions yielded 140% and 170% of biomass and lipids respectively over autotrophic growth.¹¹³ *Arthrospira platensis* reached a biomass concentration of 1.3 g/L when grown in glucose and under continuous lightning.¹¹⁰

Mass production: current technologies

Open (raceway) ponds

The industrial production of microalgae can be achieved with two types of culture

systems: open systems (raceway ponds – Figure 3) and closed systems (PBRs – photobioreactors).¹¹⁴ Open bioreactors offer the most economic aspect of microalgae culture with respect to constructions, maintenance and operation although, issues such as contamination, evaporation and land requirements persist. On the other hand, closed PBRs offer a more convenient approach to deal with evaporation and contamination but constructing closed culture vessels is costly.¹¹⁵

In the choice of culture systems, it is not only the microalgae strain that is important but also the reason for the culture. In case of biofuel production, culture should preferably rely solely on freely available sunlight irrespective of weather conditions to be economically viable.¹² Many factors have been identified as potential threats to microalgae culture such as light source, nutrients, temperature and others as discussed in the *Nutrition and culture conditions* section. For pilot-scale and commercial scale productions, raceway ponds remain the easiest and most applicable option due to the ease of setup.¹¹⁶ Raceway ponds basically consist of a closed and looped channel that is driven by a paddle wheel while the liquid flow is guided by baffles.¹² The whole system is exposed to sunlight and the paddle wheel operates to prevent the occurrence of sedimentation. Due to their simple assembly, raceway ponds can be easily scaled up.¹¹⁷ The supply of CO₂ is an excellent method of aeration and helps increase biomass production.¹¹⁸ These ponds are usually shallow to allow



Figure 3. *Spirulina* culture in open raceway ponds at Earthrise® company, US. (Permission granted for use of photo).

light penetration.¹¹⁵ The set up of such ponds does not necessitate fertile lands which is a strong point. A different type of open pond is the circular pond that operates with a rotating arm that mixes the culture and is very shallow (around 5cm). They are still used in Japan, Taiwan and Indonesia.¹¹⁹ However, these types of reactors are susceptible to contaminants and also result in low biomass productions.¹²⁰

Closed photobioreactors

Due to low productions and contaminations, closed PBR systems were created including tubular PBR, flat-plate bioreactor and column PBRs.¹²¹ Tubular PBRs such as stirred tank and bubble column are suitable systems used to reduce contamination and improve biomass productions. Mixing is achieved through the bubbling of CO₂ at the bottom of the vessel via air pumps.¹²² This type of PBR does allow control over culture conditions such as good mixing, high photosynthetic efficiency and low energy consumption but is limited to heterotrophic microalgae and this is not desired in microalgae based biofuels.¹¹⁵ Another challenge with this type of PBR is the difficulty to scale up, especially in vertical tubular PBR where costs are required for support systems and the high quality construction materials.¹²³

Tubular PBRs are designed in a way so as to reduce the light path and thus increase the availability of light to the microalgae cells. Additionally, modulating the pipe diameter is also crucial as larger pipes would provide higher liquid velocity which could potentially damage cells and the opposite will result in poor biomass production.^{124,125} Flat plate PBRs are used for large scale production of microalgae outdoors and indoors due to the high illumination surface area and ease for scaling up compared to tubular PBRs.¹²³ However, in this culture system it is difficult to control the temperature and is also subject to bio-fouling.¹²⁶ Column PBRs offer a high mass transfer capacity, are easy to sterilize and provide good mixing with acceptable shearing forces. However, the costs associated in the material for construction is very high and uses a lot of energy when operating making it less desirable.¹¹⁵

Biomass harvesting

Harvesting microalgae post culture is a very difficult task due to the size of the cells. It is estimated that at least a quarter of the production costs is incurred from the harvesting processing as they are mostly energy driven or require costly chemicals.¹²⁰ There various harvesting techniques that are currently available and/or in devel-

opment which consist of physical, chemical, flocculation, bio-flocculation and electrophoretic methods.¹²⁷ The main purpose of these processes is to dewater the microalgae slurries.¹²⁸ Sedimentation is a physical technique that works on gravitational force. The cells sediment based on density differences and work best in sedimentation tanks as the bottom area forms an inverted conical shape. However, this technique is a very slow process as microalgae cultures share similar densities to water.¹²⁹ It took 2h for *S. dimorphus* and *Chlorella vulgaris* to reach a biomass recovery of 80% and 55% respectively.¹³⁰ Similar results were obtained with *C. fusiformis* and *Nannochloropsis* sp. after a settling time of 24h.¹³¹

Filtration is another technique that allows rapid flow of culture through a porous membrane (pore size \approx 0.001 to 10 μ m) and the cells are retained. The recovery rates with filtration are very high but the process is also very costly.¹¹¹ Centrifugation is a method which operates on recovering solids from liquids through centrifugal force by high speed spinning. Biomass recovery rates can reach above 95% with centrifugation but this technique as well is very expensive.^{132,133} Flocculation consists of coagulating microalgae cells by flocculants agents such as chitosan.¹³⁴ This functions by the neutralization of the charges on the cells and the reduction of the intercellular repulsion forces leading to the cells to aggregate.¹³⁵ Successful harvesting of *Chlorella vulgaris* was reported with chitosan.¹³⁶ This technique depends on various parameters such as flocculant used, algae species and cell size.¹³⁷ Bio-flocculation is another form of flocculation where flocculating microalgae are used to concentrate non-flocculating microalgae with examples such as *Ankistrodesmus falcatus*, *Scenedesmus obliquus* and *Tetraselmis suecica*.¹³⁸ Bio-flocculation can also involve microorganisms other than microalgae such as *Peranema trichophorum*.¹³⁹ Harvesting techniques should be chosen according to costing involved and the end product desired.

Biomass conversion

Following biomass collection, the latter is subjected to downstream processing which consists of cell disruption to release the components of interest such as lipids and carbohydrates for biofuels production.¹⁴⁰ Pretreatment methods include sonication, microwaves, bead beating and freezing.¹⁴¹ On large scales, such pretreatment methods would preferably be chemical hydrolysis as the chemicals used are more economical than ultrasonication or freeze-thawing.¹⁴¹⁻¹⁴³ Acid hydrolysis of *S. obliqu-*

us yielded a sugar concentration of 95.6% making it a single step pretreatment requirement.¹⁴¹ Another study with *Tribonema* sp. gave a carbohydrate yield of 81.48% upon acid hydrolysis.⁹⁴ Acid hydrolysis is the better technique considering that microalgae do not possess cell walls compared to lignocellulosic biomass.^{86,144} There are 3 main types of post pretreatment processes used to convert microalgae biomass into suitable fuels: chemical, biochemical and thermochemical conversion.^{17,145} The chemical and biochemical pathways are used primarily for the production of biodiesel and bioethanol respectively.

The chemical process refers to the transesterification of triglycerides to methyl esters. This reaction is very similar to that encountered in the biodiesel production from plants. The triglycerides are abundant in microalgae in the form of lipids and fatty acids which normally serve as source of energy and storage products.¹⁷ The oil content of microalgae ranges from 30-70% of dry weight as it varies from species to species.¹² Triglycerides of interest would be those with chain lengths of C15-C22 and low levels of unsaturation. Microalgae can synthesize hydrocarbons that possess straight chains and this improves the cetane number of the resulting biodiesel. However, due to isomerism, these hydrocarbons can be affected by cold weather and thus it is most likely that biodiesel be used for blending with petroleum fuels.¹³⁰ As it is, biodiesel and diesel have similar properties such as cetane number, flash point and viscosity.^{146,147} Additionally, crude microalgae oil contains high levels of P, N and metals which may affect the resulting biodiesel such as NOx emissions. Further research should be oriented towards the purification of the crude oil before biodiesel productions.¹³⁰ *S. obliquus* and *S. platensis* were investigated as a candidate for biodiesel and the characteristics of the oil produced was in conformation to the biodiesel standard specifications.^{148,149}

The biochemical process is mainly dedicated to the production of bioethanol through fermentation. Carbohydrates such as sucrose are first hydrolyzed to release glucose and fructose by the activity of the enzyme invertase. Then, the fermentation of the simple sugars, glucose and fructose, is performed by the yeast such as *Saccharomyces cerevisiae* to produce ethanol.¹⁷ During the early 2000s, the lack of suitable microorganisms for industrial fermentation was already an issue and engineering bacteria such as *Escherichia coli* and *Zymomonas mobilis* was then put forward for efficient fermentation of sugars.¹⁵⁰

Microalgae hydrolysates have been suc-

cessfully fermented to produce ethanol at a concentration of 8.55 g/L.⁸⁶ The joint production of biofuels is also feasible as microalgae contain both carbohydrates and lipids. Depending on microalgae used, high levels of both biofuels can be obtained. The filamentous microalgae *Tribonema* sp. was successfully used to produce both biodiesel and bioethanol.⁹⁴ Bioethanol was also produced from *Chlorella vulgaris* at a yield of 89% using continuous immobilized yeast fermentation.⁹⁶

Biomass processing

Thermochemical processing of microalgae biomass is divided into 4 categories: liquefaction, torrefaction (combustion), pyrolysis and gasification.¹⁴⁵ Liquefaction or hydrothermal liquefaction (HTL) is the production of bio-oil by the reaction of the biomass in water at temperatures ranging between 200-300°C and at high pressure with the possible input of catalyst. The advantage of this process is that it does not require dewatering the algal biomass.¹⁵¹ The biomass is converted into liquefied products following physical and chemical changes in a complex sequence.⁷⁶ HTL of *Nannochloropsis* sp. at 250°C yielded a bio-oil yield of 30.0% by weight with the use of nano-Ni/SiO₂ as catalyst.¹⁵¹

During torrefaction, the microalgae biomass is degraded at temperatures ranging from 200-300°C in inert environments for a few minutes to a few hours.^{77,145} This process ensures that the microalgae gains in calorific values and yields a charry solid. Investigations on the effects of this technique are already available.¹⁵² Pyrolysis is similar to torrefaction but works at higher temperatures such as 400-600°C. However, the temperature varies with respect to the time allocated to the process. The main products are bio-oil, charcoal and gaseous fractions.¹⁵³ Bio-oil was produced at 57.9% during flash pyrolysis of *Chlorella protothecoides* at 450°C.¹⁵⁴ Pyrolysis of *S. platensis* produced bio-oil at a yield of 29% at 500°C compared to 23% at 350°C indicating temperature differences affect the yield.¹⁵⁵

Gasification is the conversion of microalgae onto H₂, CO, CH₄ and other combustible gases. This technology can be divided into two types: conventional gasification and supercritical water gasification.¹⁴⁵ Gasification of microalgae *Nannochloropsis gaditana* was performed successfully at an efficiency of 97.4% with the production of hydrogen, methane and carbon dioxide at 52%, 17.9% and 23% respectively.¹⁵⁶ Despite the good production values, it should be noted that such operations require high energy input to generate

high temperatures and thus can be regarded as economically not viable.

Waste water for improved biofuel production

The use of microalgae for waste water treatment can be considered as phytoremediation as it is the decontamination of waste-waters of pollutants or removal of xenobiotics. Waste waters can be in the form of municipal waste waters, agricultural rejects, and industrial discharges. Excessive nutrients can be removed by growing microalgae or macroalgae.⁸⁴ Medium containing fermented swine urine as waste-water was used to grow *Scenedesmus* sp. for phycoremediation. The growth rate was improved by 3 folds and secondary metabolite production was also increased (astaxanthin by 2.8 folds and β-carotene by more than 5 folds).¹⁵⁷ *Nannochloropsis salina* was tested as a candidate to decompose organic matter in anaerobic digestion. Maximum biomass production was evaluated at 92 mg/L/d when 6% of effluent was used.¹⁸ The use of waste waters is an appealing alternative source of nutrients for microalgae culture but specific conditions should be met for the culture to be successful. The nitrogen concentration is crucial in microalgae culture as its presence in high quantities ensures the uptake of phosphorus.¹⁰⁶ The culture of *Chlorella vulgaris* in agricultural waste products under mixotrophic conditions yielded a dry weight of 2.62g/L and a lipid yield of 0.86g/L.¹¹³ Phycoremediation was also successful with *Chlamydomonas debaryana* in wastewaters where maximum lipid production was quantified at 87.5mg/L/day.¹⁵⁸ Additionally, waste waters used as blends are cost-effective sources of nutrients suitable for microalgae culture.¹⁵⁹ This can help reduce the capital investment or use the investments for other purposes.

Economic perspective of microalgae culture and potential improvements

The concept of using microalgae for biofuels production is very appealing to counter the use of fossil fuels and plant biomass. However, for the time being, the production status is very limited on the industrial scale due to the high production costs associated with the culture and processing of the biomass.¹²⁰ The simplest of open pond culture systems would cost a minimum of \$100,000 including all of the processing chain.¹⁶⁰ When compared, open ponds are way cheaper to build and maintained than photobioreactors but on the other hand, the yield of biomass harvested from photobioreactors is higher.¹⁶¹ An eval-

uation of a hierarchical control strategy for microalgae culture using photobioreactors has been performed and the cultivation cost was found to reach 100€/kg and a lowered CO₂ loss of 167g/d.¹⁶²

On large scale, microalgae culture costs can still be lowered with the use of inorganic carbon sources. The incorporation of flue gases as CO₂ source in photoautotrophy cultures would be a better choice because the carbon is not delivered to the atmosphere.¹⁶³ A Profit and Loss analysis was conducted with *Haematococcus pluvialis* where production costs dropped by 18% when water was recycled, solar energy was used as energy source and CO₂ flue gas was used.¹⁶⁴ The inclusion of waste waters as a source of nutrients is also a beneficial way of lowering costs as reviewed in the *Waste water for improved biofuel production* section. Waste waters contain nitrogen and phosphorus which are the major nutrients for microalgae thus making such a source relevant.

The major economical issues arise with the downstream processes, starting with the harvesting techniques.¹⁴⁰ Due to the negative charge, small cell size and motility, different techniques are currently used to harvest microalgae such as centrifugation, sedimentation, flocculation and filtration, or combinations of them.¹⁶⁴ Harvesting microalgal biomass may alone contribute up to 20-30% and even up to 50% of the costs in the production chain.^{165,166} Low cost techniques are currently being investigated such as the use of biological flocculants (discussed in the previous sections). At this point, one should bear in mind that downstream processing differs with respect to the expected end product(s).¹¹⁷ If the culture is solely for biofuels production, the costs will be less than a culture being performed to generate value-added products such as astaxanthin which will require very expensive processes and equipment.

Genetically engineered microalgae for improved biofuels production

Modifying microalgae at the genetic levels can be very useful as it may increase the potential of certain strains to produce more lipids or carbohydrates for biofuels production.¹⁶⁷ For lipids, higher rates of accumulation are being researched through changes in the biochemical pathways.¹⁶⁸ Some enzymes are being over expressed such as the malic enzyme in microalgae *Phaeodactylum tricoratum* where the transgenic version yielded a 2.5-fold of total lipids compared to the wild strain.¹⁶⁹ Similar results were obtained with *Schizochytrium* sp. where over-expression of the gene superoxide dismutase (SOD1)

yielded 32.9% higher lipids content than the wild type.¹⁷⁰ Another research with transgenic *Chlorella sorokiniana* and *Chlorella vulgaris* has shown that over-expression of gene carbonic anhydrase yielded 2.2-fold of lipid accumulation than that of their respective wild types.¹⁶⁸

In the case of bioethanol, some microalgae directly synthesize it as a metabolite and research is ongoing in trying to increase the yield.¹⁷¹ The insertion of certain genes at specific regions can be very advantageous. This was demonstrated when aldolase was co-over expressed with pyruvate decarboxylase and alcohol dehydrogenase in cyanobacterium *Synechocystis* PCC 6803 yielding 69% more ethanol and a higher biomass of 10.1%.¹⁷² An important aspect of culturing bioethanol producing microalgae is their tolerance to the ethanol as high concentrations can be toxic to the cells. One example is recombinant cyanobacteria *Synechocystis* UL 030, which was engineered with 2 cassettes of genes per genome to become more tolerant to ethanol than its wild type.¹⁷³

Such experiments and findings demonstrate the capacity of transgenic microalgae in the production of biodiesel and bioethanol. However, even though this application seems appealing, great care should be taken when handling genetically modified organisms as they do not exist naturally. Cyanobacteria have the potential of transferring genes horizontally with unrelated microbes and this will require thorough assessments to evaluate the harm caused to the environment in case they manage to thrive there.¹⁷⁴ Therefore, culturing modified microalgae will require intense supervision to avoid their introduction into surrounding ecosystems.

Exploiting microalgae and residual biomass for other applications

Feed production

With the production of 3rd generation biofuels, other appealing applications of microalgae are being researched as potential sources of phytochemicals, valuable chemicals and food/feed additives.¹⁷⁵ If compared to plants, microalgae are photosynthetic microorganisms that lack specialized organs such as leaves, vascular tissue, etc. as plants do.¹⁶ However, when it comes to cellular composition, microalgae can also synthesize lipids, proteins and carbohydrates as well as numerous bioproducts such as vitamins, pigments and antioxidants.⁹² Following extraction of lipids and carbohydrates from biofuels production, there is the output of residual biomass which would consist mainly of proteins and potentially, other molecules in the form of

vitamins, pigments and antioxidants.

Research with *Scenedesmus obliquus* has shown that the residual biomass can be used as feed for brine shrimp after lipid extraction for biodiesel production. The average fresh weight of the shrimp increased from 3.8g to 4.7g when residual biomass applied was increased from 0.01g/L to 0.4g/L.¹⁴⁸ In aquaculture practices, using microalgae as feed has been an ongoing practice for the past decades. Microalgae are important sources of protein likely to replace soybean meal, fish meal and rice bran in feed formulations.⁹⁰ With issues such as fish meal being expensive and not always readily available, other sources of feed need to be investigated and microalgae was identified as a potential source.¹⁴ Microalgae are an excellent source of feed for fish larvae, molluscs and crustaceans. The quality of the microalgae has a direct impact on the growth rate and reproduction rates of *Artemia* (brine shrimp).¹⁷⁶ *Nannochloropsis* sp, *Pavlova lutheri* and *Isochrysis* sp. are some of the microalgae that are fed to brine shrimp, which are in turn fed to advanced stages of fish larvae and crustaceans.^{177,178}

However, it is also essential for microalgae based feed to contain fatty acids such as linoleic acid (18:2w6), linolenic acid (18:3w3), eicosapentaenoic acid (EPA, 20:5w-3), arachidonic acid (ARA, 20:4w6) and docosahexaenoic acid (DHA, 22:6w-3) as well as some essential amino acids such as tryptophan considering that shrimps and fish cannot synthesize them.¹⁷⁹ Lack of these components in diets may result in poor growth and subsequently in death. Such important fatty acids accumulate in fish/crustaceans through the food chain.¹⁸⁰ Microalgae are excellent sources of such fatty acids. Investigation using microalgae *Porphyridium cruentum* demonstrated that EPA was produced at a peak of 13.1%, ARA at 30.5%, linoleic acid at 27.6% and linolenic acid at 0.4% (under various conditions).¹⁸¹ Microalgae are also cultured for other marine organisms. Diatoms such as *Navicula* sp. are fed to juvenile abalone, which are highly appreciated as a seafood delicacy in Asian countries.^{178,182} Microalgae can also serve as ingredients for feed additive production. Incorporating *Spirulina platensis* to the feed of *Oreochromis niloticus* (Nile tilapia) as 10g/kg diet improved the growth performance and immunity of the fish.¹⁸³

Bioactive molecules production

Microalgae have the ability to produce various biomolecules such as astaxanthin, lutein, beta-carotene, chlorophyll as well as fatty acids among many others.¹⁴ Among

the bioactive compounds, some tend to have antimicrobial properties. On the global market, the number of effective antimicrobial drugs is gradually decreasing with the increase in antimicrobial resistance.^{184,185} This fact has been known for a long time and many mechanisms allowing the antibiotic resistance to occur in bacteria have been identified.¹⁸⁶ Microalgae such as *Chroococcus dispersus*, *Chlamydomonas reinhardtii* and *Chlorella vulgaris* are known to exhibit antimicrobial activities.¹⁸⁷ The major and valuable secondary metabolite productions in *Scenedesmus* sp. are astaxanthin and β -carotene.¹⁵⁷ Astaxanthin is also produced from *H. pluvialis* at a rate of 1.5-3% of the dry weight and is considered as high-value carotenoid.¹⁶³ It provides very good protection for the membrane phospholipids. Additionally, this molecule offers health benefits such as anticancer, anti-inflammatory and immune-modulating functions.¹⁸⁸ *D. salina* production is currently at 1200 tons per annum in Australia. Israel, USA and Japan for the production of various valuable goods such as β -carotene.¹⁸⁹ At a closer look, biofuels production from microalgae can simultaneously lead to various business opportunities as they contain such value-added products.¹²¹

Conclusions

Biofuels such as bioethanol and biodiesel are predominantly produced from biomass for the transport sector. Their introduction as a source of energy was initiated from the controversies associated with the use of fossil fuels such as carbon dioxide emission, global warming and the fact that they are a finite source of energy. The production of these biofuels from sugarcane, corn, soybean and palm is already at the industrial scale. Their use alleviates the issues associated with gasoline and diesel but they are in turn subject competition for food, land for agriculture and increasing food prices. The same problems were identified when biofuels were being produced from non-food crops (*Arundo donax* and *Jatropha curcas*) and attempts are being made to improve processes for higher yields.

In the mean time, microalgae were found as a potential source of same biofuels. These algae have a much higher growth rate compared to plants and need only 2Mha of land to produce 136900L/ha of biodiesel. The culture of microalgae can be done in open ponds, closed systems and photobioreactors but need to be economically viable. Microalgae do not require fertile land and thus does not compete for food

production. Other advantages of microalgae are their ability to produce high yields of lipids and carbohydrates when subjected to stress conditions and to thrive in waste waters. For liquid biofuels, microalgae biomass is processed through two main processes such as chemical and biochemical techniques which yield biodiesel and ethanol respectively. However, the use of microalgae for biofuels is limited due to the costs associated with the implementation and production. Improvements in the biomass processing techniques and culture parameters need to be brought to make this field relevant and economically feasible.

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