Chapter 17
Microalgal Feedstock for Bioenergy: Opportunities and Challenges

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Abstract The utilization of algal feedstock for bioenergy can be considered as one of the greatest challenges for biosystems engineering in the near future. Some species of microalgae show high potential for oil accumulation and further utilization of its biomass for biogas generation, pyrolysis, ethanol production, and even as fertilizer. Microalgae can utilize CO_2 as carbon source and can also be grown on nonagricultural environments, such as wastewater facilities, industrial effluents, freshwater, and marine water habitats. The vast research field on microalgae engineering is due to the facts that it can be a source of energy and act as an air and water pollutants removal. There have been considerable advances in engineering its growth, in bioreactor designs, and on lipid accumulation due to chemical, biochemical, and genetic studies. Despite that, there are still some fundamental processing aspects that are considered challenges, either economical, ecological, or technical, such as biomass harvesting and the competition with the higher value products produced from algae, as proteins.

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17.1 Introduction

The pursue of alternative sources for energy in the new century is due to the scarcity of fossil fuels in the near future, i.e., energy security reasons, and also the concern with the environment. According to the Intergovernmental Panel on Climate Change (IPCC), the accelerated production of carbon dioxide as a result of human activity is a major factor which contributes to the greenhouse effect (Houghton et al. 2001). The history of biofuels, which have been considered a green alternative for fossil fuels, has been changing much for the past 40 years. There were an ethanol boom with Brazil and the United States (Ribeiro and Younes-Ibrahim 2001; Goldemberg et al. 2004) and a huge interest in producing biodiesel from oleaginous plants in the last decades (Demirbas 2008; Pousa et al. 2007). Despite these being designed as green alternatives, recent studies imply that ethanol and biodiesel produced from plant feedstocks do not match several criteria for sustainability (Hoekman 2009). The large acreage of corn for ethanol production in the United States, for example, has raised concerns among specialists regarding pollution from pesticides and fertilizers, reduction of biodiversity, soil erosion, and a shift on the equilibrium on the food supply chain (Fargione et al. 2008, 2010; Hill et al. 2009).

An alternative showing promising results are known as second-generation biofuels, i.e., biofuels produced from lignocellulosic residues (Sun and Cheng 2002). These are still being developed and are based on the utilization of sugar monomers released from agro-residual biomass hydrolysis and on the production of biogas from biomass controlled combustion (Hendriks and Zeeman 2009). Despite these efforts are considerable and important for supplying clean energy to human society, microbes have been considered as one of the new potential sources of energy harvesting (Xia et al. 2011; Huang et al. 2009; Li et al. 2008; Millati et al. 2005; Illman et al. 2000; Ratledge and Wilkinson 1988). In the group of microbes, microalgae have earned much attention from the academic society for a vast number of reasons. Some of which are: the tendency of producing more biomass than terrestrial plants per unit of area, they can be produced in marginal lands, in fresh water, and in salt water ecosystems (Chisti 2007) and their non-competition with food systems, since they can be produced in areas where there is no agricultural productivity (Hill et al. 2006). Another characteristic of microalgae that can make its production more feasible and sustainable is its capacity to uptake human produced CO_2 (Benemann and Oswald 1996) as well as removing certain water pollutants (Powell et al. 2008; Munoz and Guieysse 2006).

The interest in microalgae is not something new though from 1978 to 1996, the U.S. Department of Energy funded a program to develop renewable transportation fuels from algae (Sheehan et al. 1998). Their goal was the production of biodiesel from high lipid content algae utilizing waste CO_2 from coal plants, and throughout these almost 20 years of research, there was a considerable advance in metabolism manipulation and bioprocessing engineering for algae growth. In the recent years, there was a development in the field of investigation on genetic modification for

enhancing lipid production (Rosenberg et al. 2008; Radakovits et al. 2010), as well as the studies on biochemical engineering regarding optimization of growth. Factors such as reactor configuration (Vergara-Fernández et al. 2008; Wu and Merchuk 2002, 2004), nutrient loads (Fabregas et al. 2000; Heredia-Arroyo et al. 2010; 2011), light fluxes, and others are some present in the literature.

Another key aspect regarding algae for bioenergy is the utilization of its dry biomass for biogas generation (Vergara-Fernández et al. 2008; Bohutskyi and Bouwer 2013; Mussgnug et al. 2010), for production of other fuels and even as feedstock for char as potential fertilizer (Johnson et al. 2013). Therefore, it can be seen that the trend of microalgae research nowadays is mainly focused on the conversion of algal biomass to fuels and the engineering toward optimization of cultivation methods and oil and lipid enhancing.

The purpose of this chapter is to present some technologies available in the field of growing, harvesting, and utilizing microalgae biomass, the chemical and biochemical nature of microalgae biomass, and the basic concepts of biodiesel, biogas, biohydrogen, bioethanol, and other fuels production from microalgae biomass and lipids. Alongside the technologies, the current challenges and some opportunities are presented.

17.2 Cultivation of Microalgae

As well as any other microorganism, microalgae grow in environments with its basic nutritional needs. In lab scale, there have been studies on formulating specific medias for their growth since the nineteenth century (Lourenço 2006). Basically, all the culture media for microalgae cultivation should be composed of basic macronutrients (C, H, O, N, P, S, K, Mg, Si, and Fe) and micronutrients (Mn, Mo, Co, B, V, Zn, Cu, Se, Br, and I) (Lobban 1994), as well as light and water. Grobbelaar (2004) presented the following ratio for some nutrients: $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$. There are several classifications, from which two are more useful in this chapter: into marine species, which have affinity for high concentration of salt, and freshwater species (Bilanovic et al. 2009); and the division into autotrophic, heterotrophic, and mixotrophic species, noticing that some species can grow under two or three of these regimes (Heredia-Arroyo et al. 2010).

Light administration is a key factor in both indoor and outdoor systems, affecting especially those microalgae that grow on photoautotrophic regime. For outdoor systems the most common light source is sunlight while in indoor cultivation, artificial light sources are required. Chen et al. (2011) summarized several artificial light sources, from the conventional one (with a high electricity consumption) to more engineered options, such as LED, Optical fiber excited by metal-halide lamp (OF-MH), Optical fiber excited by solar energy (OF-solar), and an option with zero electricity consumption and high operation stability, which is the LED/OF-solar combined with wind power/solar panel.

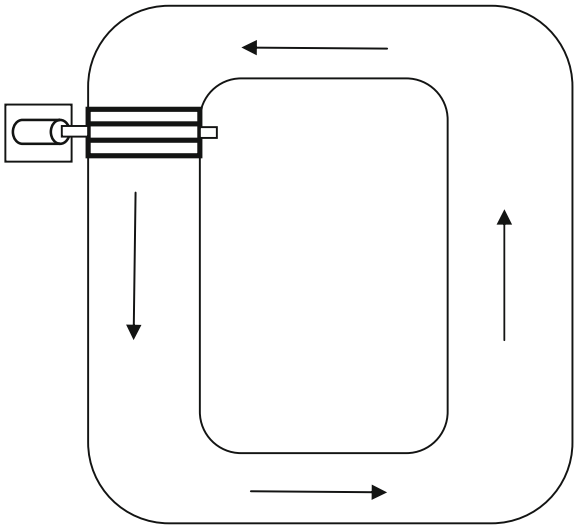

Fig. 17.1 Scheme of a Raceway pond

Producing microalgal biomass nowadays is generally more expensive than crops, although the culture media are inexpensive (Acién Fernández et al. 1999). There should also be a temperature control within 20–30 °C in most cases (Chisti 2007).

17.2.1 Large Scale Production of Photoautotrophic Microalgae

According to Chisti (2007), the only practicable ways to produce microalgae in large scale are in raceway ponds and in photobioreactors.

A raceway pond is a simple and continuous system of microalgae growth. It is consisted on a closed loop recirculation channel, with an average depth of 0.3 m, built generally with PVC, concrete, or compacted earth covered with a plastic film (Chisti 2007; Terry and Raymond 1985). The continuous flow is guided around bends and it is mixed and circulated by a paddlewheel, used also to prevent sedimentation. After its retention growth, culture broth is harvested on the completion of the circulation loop. A simple scheme of a raceway pond can be seen in Fig. 17.1.

There are also circular ponds and unstirred ponds. The main advantage of a raceway pond, when compared to a photobioreactor, is its price, having operational and building costs lower than the other does.

Photobioreactors (PBRs) are closed systems, which have the differential characteristic of enhancing light availability for each microalgae cell in the reactor (Suali and Sarbatly 2012). These are generally built with transparent materials,

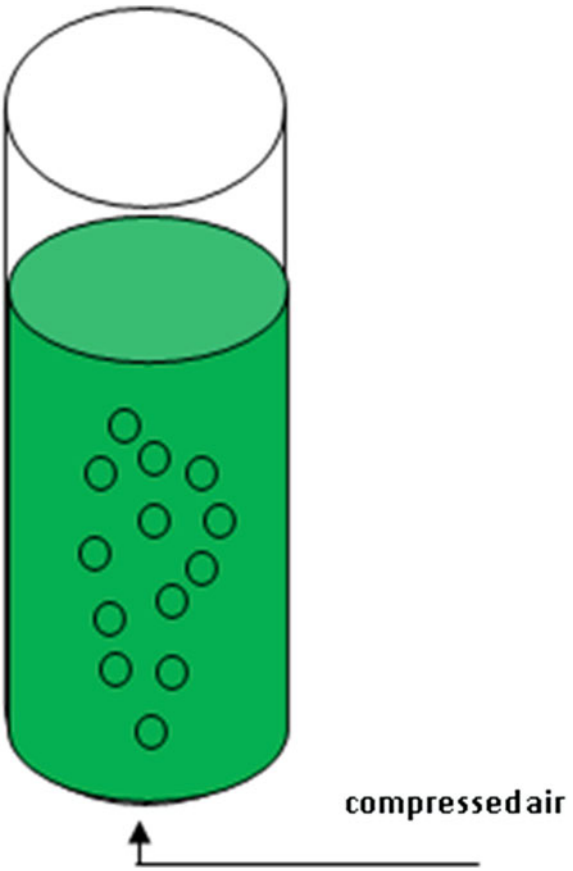

Fig. 17.2 Scheme of a cylinder photobioreactor

allowing light penetration into the culture. These are usually more expensive than raceway ponds, however, they show higher productivity (Lourenço 2006). In the literature, three types of geometry are most cited: the flat-plate, the cylinder, and the tubular.

Tubular and PBRs are constructed with transparent glass or plastic, and these sort of PBRs have been gaining attention from the academic community in the past decades. Geometrically, they can be horizontal, vertical, conical, and even inclined. Mixing can be done either by air lift or by air pumps (Chen et al. 2010). A simple scheme of a tubular PBR can be seen in Fig. 17.2.

The flat-plate bioreactors consist of airlift driven columns or rectangular tanks with a recirculation loop. In this kind of reactor, illumination is provided by an external light source or a bank of lights (Silva et al. 1987).

17.2.2 Cultivation on Heterotrophic Conditions

The so-called heterotrophic cultivation is that one utilizes organic carbon under dark conditions, as purpose for carbon sources and energy. There are a large number of organic substrates that microalgae can assimilate, such as glucose, acetate, glycerol, fructose, sucrose, lactose, galactose, and mannose, which can be derived from residue biomass, such as corn stover, wheat straw, and sugarcane bagasse hydrolysates (Chojnacka 2004).

Heredia-Arroyo et al. (2010) confirmed that heterotrophic growth could result in a higher biomass concentration, when compared to autotrophic conditions for *Chlorella protothecoides* under the conditions they studied; the oil content was similar between these two conditions though. A drawback of using heterotrophic cultivation is the possibility of bacterial contamination.

17.2.3 Cultivation of Mixotrophic and Photoheterotrophic Microalgae

Mixotrophic cultivation is the one when microalgae undergo photosynthesis and use both organic compounds and CO_2 as carbon source (Chen et al. 2011). Cultivating a microorganism under mixotrophic conditions means that this organism would be able to grow under phototrophic, heterotrophic, or both conditions.

There is also a cultivation condition known as photoheterotrophic, also known as photoorganitrophic, photoassimilation, and photometabolism. In this sort of metabolism, light is a requirement to utilize organic compounds as carbon source.

Utilizing mixotrophic and photoheterotrophic conditions to grow microalgae, with a supply of organic compounds from waste resources with zero or even negative carbon and economic costs may be a good opportunity. Some mixotrophic conditions may enhance lipid accumulation, achieving yields significantly higher than those in solely autotrophic conditions (Heredia-Arroyo et al. 2011).

A brief comparison of the characteristics of different cultivation conditions is shown (Chen et al. 2011) in Table 17.1.

17.2.4 Biomass Harvesting Techniques

The recovery of algal biomass consists on a solid–liquid separation process and may account with costs up to 30 % of the total costs in producing microalgal derived fuels (Gudin and Thepenier 1986). The traditional biomass harvesting processes are: flocculation, filtration, flotation, and centrifugal sedimentation. The most appropriate harvesting process depends on a series of factors, such as culture density, size, value of the products, and available technology (Brennan and Owende 2010).

Table 17.1 Comparison among cultivation conditions

Cultivation condition	Energy source	Carbon source	Achieved cell density	Reactor scale-up	Cost	References
Phototrophic	Light	Inorganic	Low	Open pond or PBR	Low	Chen et al. (2011)
Heterotrophic	Organic	Organic	High	Conventional fermentor	Medium	Chen et al. (2011)
Mixotrophic	Light and organic	Inorganic and organic	Medium	PBR	High	Chen et al. (2011)
Photoheterotrophic	Light	Organic	Medium	PBR	High	Chen et al. (2011)

Cell flocculation is a process in which cells are aggregated together, in order to form larger particles for settling, i.e., decanting. There are some process well established, such as chemical autoflocculation (precipitation of carbonate salts with algal cells at an alkaline pH) (Chen et al. 2011), chemical coagulation (with organic and inorganic coagulants) (Grima et al. 1994), and even combined flocculation techniques.

Filtration is known as the unit operation of separating solid particles from a suspension using a screen with a particular pore size, accumulating solids at one side, and decreasing biomass concentration on the other (Grima et al. 1994). This technique shows several advantages toward other methods, such as simplicity in cost and operation, and with some adaptations, it can achieve recovery rates of up to 89 % (Petrusevski et al. 1995). There are some operation limitations, e.g., the reduction of effectiveness when biomass is too concentrated and clogging issues (Chen et al. 2011).

Flotation is based on gravity separation in which air or gas bubbles attach to solid particles and then carry them to the liquid surface (Chen et al. 2011). Flotation can be divided in two major categories: Dissolved air flotation (DAF) and Dispersed air flotation. The main difference between these two is the bubble size; while in the first, the air bubbles are within the diameter range of 10–100 μm, the second one is based on bubbles of 700–1500 μm formed by a high speed agitator (Rubio et al. 2002).

Centrifugation is a traditional harvesting method, even though its operational costs can be high enough to make it unfeasible. It is a quick and effective method, achieving recovery rates of up to 90 % within 2–5 min (Chen et al. 2011). Depending on the situation, it may not be adequate, since high shear forces could damage cell wall.

17.2.5 Oil Extraction Techniques

Oil extraction from microalgal biomass is usually an energy demanding process, due to, among other factors, the requirement of biomass dewatering. The costs of harvesting and dewatering can achieve costs up to 30 % of the total algal biodiesel

Table 17.2 Comparison among biomass drying processes

Method	Advantages	Disadvantages	References
Drum-drying	Fast, efficient	High costs	Chen et al. (2010); Becker (1994)
Spray-drying	Fast, efficient	High costs	Chen et al. (2010); Becker (1994)
Sun-drying	Cheap	Slow, weather dependent	Chen et al. (2010); Becker (1994)
Solar-drying	Cheap	Weather dependent	Chen et al. (2010); Becker (1994)
Cross-flow-drying	Moderate rate and costs	Electricity costs	Chen et al. (2010); Becker (1994)
Vacuum-shelf-drying	Gentle process	High costs	Chen et al. (2010); Becker (1994)
Freeze-drying	Gentle process	Slow, high costs	Chen et al. (2010); Becker (1994)

production (Tampier et al. 2009). The most economically viable way would be natural drying, with solar and wind energy, however this would be a weather dependent process, which could make oil production seasonal. There are a few common drying methods, as well presented by Chen et al. (2010) and Becker (1994) in Table 17.2.

A widely used method for extracting oil from microalgal biomass is using organic solvents. It is a common pathway for extracting oil from oleaginous plants and it has been used for microalgae in most cited cases as well (Grima et al. 1994). The ideal organic solvent should have some characteristics: it has to match the lipid polarity in the cells, it should be cheap, easy to remove, present low to zero toxicity, insoluble in water, recyclable, and efficient in dissolving some targeted components (Chen et al. 2010).

Even though chloroform has high risks of toxicity and flammability, it is a very common solvent used in lipid extraction. Chloroform is able to extract hydrocarbons, carotenoids, chlorophylls (source of the green color of algal oil), sterols, triacylglycerols, wax esters, fatty alcohols, aldehydes, and free fatty acids (Chen et al. 2010). A traditional method which combines chloroform with methanol at a 2:1 v/v ratio is one of the most used in the published studies (Folch et al. 1957). Attempts in utilizing other solvents, such as ethanol, 1-butanol, hexane, isopropanol, and hexane have also been studied (Grima et al. 1994; Medina et al. 1998; Cartens et al. 1996; Nagle and Lemke 1990).

In order to optimize solvent extraction, some mechanical and physic-chemical approaches have been studied to disrupt microalgal cell wall. These include: autoclaving, microwave, sonication, bead-beating, osmotic shock, and cell grinding, i.e., "blending," freeze-press and enzymatic and chemical lysis. Such methods will not be discussed in this chapter, but they are very well presented by Chisti and Moo-Young (1986).

Two new techniques in extracting lipids are the supercritical fluid extraction (SFE) and the subcritical water extraction (SWE). The idea behind SFE is the utilization of supercritical thermodynamic properties, such as better diffusivity and decreased viscosity, which improve diffusion rates through solid materials, aiming higher extraction efficiencies (Anklam et al. 1998). On the other hand, the principle of SWE is the utilization of water under subcritical conditions, which decreases its polarity, improving solubility of nonpolar organic compounds (Chen et al. 2010).

17.3 Microalgal Biomass Utilization

The composition of microalgal biomass varies according to several factors: according to the species, to the growth conditions, temperature, Carbon to Nitrogen ratio, and others (Volkman et al. 1989). This section will describe some technologies used in harvesting bioenergy from microalgal biomass.

There are a few most studied species used in the bioenergy field, such as *Chlorella* sp. (Illman et al. 2000; Heredia-Arroyo et al. 2011; Wang et al. 2010; Liang et al. 2009), *Dunaliella* sp. (Tang et al. 2011; Shuping et al. 2010; Zou et al. 2009; Minowa et al. 1995), *Nannochloris* sp. (Takagi et al. 2000; Demirbas and Fatih Demirbas 2011; Hsieh and Wu 2009), *Parietochloris incisa* (Bigogno et al. 2002), and a few others. Among these, *Nannochloris* and *Dunaliella* are marine microalgae and some *Chlorella* sp. are as well.

17.3.1 Biodiesel from Microalgal Oils

One of the key points in investing technology to achieve a sustainable biodiesel production pathway from nonedible microalgal oil is its high productivity, achieving numbers as high as 5000–100,000 L ha^{-1} a^{-1} (Levine et al. 2010).

As well described by Qiul et al. (2011), production of biodiesel from microalgae consists of a series of steps: lipid extraction, removal of solvent, catalyzed transesterification, and purification. There are also a few other processes, such as hydrolysis followed by esterification (also known as hydroesterification), in situ transesterification, and supercritical transesterification.

The well-known reaction of lipids transesterification consists of a reaction of a fatty ester with an alcohol, in order to form fatty acid alkyl esters and glycerol (Ma and Hanna 1999). This reaction under normal conditions of pressure and moderate temperatures and reaction times usually requires a catalyst, which can be alkaline, acid, or enzymatic (Meng et al. 2009).

Microalgal oil has some characteristics that are not desirable in the biodiesel production: it usually has a high free fatty acid value (Miao and Wu 2006) and it also contains a high degree of polyunsaturated fatty acids (PUFA) when compared to vegetable oils (Chisti 2007). A major implication on having a high free fatty

acid value is making the alkali catalysis, which is the cheapest one, unviable (Lotero et al. 2005). Having a high degree of PUFA makes it more susceptible to oxidation, thus limiting conditions of storage (Chisti 2007).

There are a few publications about the so-called in situ transesterification. This process consists of simultaneously extracting and transesterifing the lipids to produce biodiesel. The biomass has to be dewatered, since water can act as an inhibitor in this process (Chen et al. 2010). This can be a promising strategy, since costs are lowered due to the removal of a step in the whole production.

Due to the fact of having high free fatty acid indexes, the alternative of producing biodiesel from microalgal oils through hydroesterification has also been considered. Hydroesterification is consisted of a hydrolysis followed by an esterification (Diaz et al. 2013; Reyes et al. 2012). Very little has been done yet utilizing microalgal lipids, even though it may be a good alternative for research. Reyes et al. (2012) utilized an autoclave reactor at 250 °C for the hydrolysis reaction and niobium powder for the esterification, achieving conversion rates, i.e., formation of methyl esters up to 91.7 %.

There has been a trend in this field of study using supercritical conditions. Patil et al. (2011) studied the optimization of a single-step supercritical process for simultaneous process for simultaneous extraction and transesterification of wet algal biomass, using methanol as alcohol. They present some advantages of using supercritical conditions, such as that they use modest temperatures, the high rate of production, and the final product price, which, according to the authors, is even lower than the biodiesel produced from traditional transesterification.

Up-scale processes have been studied as well. Li et al. (2007) presented the results of utilizing bioreactors with up to 11,000 L at a biodiesel production rate of 6.24 g L^{-1} and conversions up to 98.15 %. They used immobilized lipase from *Candidia* sp. and the microalgal species was *C. protothecoides.*

Biodiesel from microalgal oils has some advantages when compared to petroleum diesel: it can be a totally renewable and biodegradable fuel, a low carbon footprint, it has low levels of toxicity, and it contains reduced levels of particulates, carbon monoxide, hydrocarbons, and SO_x (Brennan and Owende 2010). Another key point that may lead biodiesel from microalgal oils to a commercial process is its low freezing point and its high energy densities, making it an interesting alternative for the aviation industry (Chisti 2010).

17.3.2 Biogas from Microalgal Biomass

In order to make microalgae a more sustainable source for bioenergy, there must be a use for its residual biomass, i.e., the biomass in which higher value products were removed, such as lipids and proteins. The high productivities of microalgae may release high amount of nitrogen and phosphate into the environment, which would shift the bioenergy harvesting from microalgae toward an unsustainable position. A process that could solve this issue is the anaerobic digestion, converting biomass to

biogas, recovering more usable energy from cell walls. Anaerobic digestion is the conversion of organic wastes into biogas, which consists of methane and CO_2, with traces of other compounds, such as H_2S (Bridgwater 2008).

Theoretically, there is more energy to be harvested through anaerobic digestion, producing a mixture rich in methane, than from lipid extraction (Sialve et al. 2009). So far, it is still a research field with very little work done and published. Only small-scale experiments have been reported achieving efficiencies in the range of 20–80 % (Zamalloa et al. 2011).

Sialve et al. (2009) identified a few challenges in digesting microalgae: the biodegrability can be low depending on the biochemical composition and on the nature of the cell wal,; which may result in ammonia release leading to toxicity in cases with high cellular protein content and in inhibition of the process by sodium, when marine species are considered.

There are a few key points that make microalgal biomass an interesting opportunity for investing in anaerobic digestion. Besides carbon, nitrogen, and phosphorus, there are nutrients in lower concentration, such as iron, cobalt, and zinc, which are able to stimulate methanogenesis (Speece 1996). The theoretical methane production increases with higher lipid content, since lipids are energy condensed structures (Angelidaki and Sanders 2004).

Sialve et al. (2009) calculated the methane potential and ammonia release from anaerobic digestion of several different species of microalgae using data from (Becker 2004). The results of these researchers are presented in Table 17.3.

The quantity and the quality of biogas generated are dependent upon the biomass composition, pH, temperature, solid retention time, hydraulic retention time, and loading rate (Singh and Olsen 2011).

17.3.3 Ethanol from Microalgal Biomass

There are three possible pathways for producing ethanol from microalgae. Algae can assimilate considerable amounts of starch and cellulose, which can be convertible to fermentable sugars. These can be fermented to produce ethanol using a yeast strain, for example. Some species can also produce ethanol during the dark fermentation methabolic pathway; the third possible process is to generate genetic engineering microalgae to produce ethanol directly (John et al. 2011).

Starch is stored in microalgal cells and can be extracted from biomass at regular intervals from photobioreactors or open ponds through mechanical processes or by dissolution of cell walls through enzymatic reactions. This starch goes through solvent extraction and then used for microbial fermentation (John et al. 2011). Once again, the biomass composition is a key point to achieve high yield on this sort of fuel. It has been reported that *C. vulgaris* is a good source for ethanol fermentation, due to the high starch content, of around 37 % dry weight, achieving conversion efficiencies of up to 65 % (Hirano et al. 1997). Following well-known procedures, Harun et al. (2010) investigated the feasibility of producing ethanol

Table 17.3 Biomass composition of several different species of microalgae with CH_4 and $N–NH_3$ productivity (VS = Volatile solids)

Species	Protein (%)	Lipid (%)	Carbohydrate (%)	CH_4 (L g^{-1} VS)	$N–NH_3$ (mg g^{-1} VS)	References
Euglena gracilis	39–61	14–20	14–18	0.53–0.8	54.3–84.9	Sialve et al. (2009)
Chlamydomonas Reinhardtii	48	21	17	0.69	44.7	Sialve et al. (2009)
Chlorella pyrenoidosa	57	2	26	0.8	53.1	Sialve et al. (2009)
Chlorella vulgaris	51–58	14–22	12–17	0.63–0.79	47.5–54.0	Sialve et al. (2009)
Dunaliella salina	57	6	32	0.68	53.1	Sialve et al. (2009)
Spirulina maxima	60–71	6–7	13–16	0.63–0.74	55.9–66.1	Sialve et al. (2009)
Spirulina platensis	46–63	4–9	8–14	0.47–0.69	42.8–58.7	Sialve et al. (2009)
Scenedesmus obliquus	50–56	12–14	10–17	0.59–0.69	46.6–42.2	Sialve et al. (2009)

from *Chlorococum* sp. biomass and achieved yields as high as 3.8 g/L using a 10 g/L substrate solution, through fermentation by *Saccharomyces bayanus*.

Besides starch, microalgae can also accumulate cellulose in their cell walls, as a structural polysaccharide. This is a common characteristic among green algae (John et al. 2011). Cellulose can be hydrolyzed into its monomers, i.e., glucose monosugars, and further fermented to ethanol. A huge advantage when comparing biomass residues from algae when to plant materials is the inexistence of lignin in algae, therefore, reducing energy costs and making ethanol production from cellulose a more feasible process.

A second possible pathway is through the metabolic pathway called dark fermentation. In absence of light and in presence of oxygen, microalgae usually maintain their life by consuming starch or glycogen; however, if oxygen is also not available, the oxidative reaction of starch is incomplete, and several other products are formed, such as hydrogen gas, carbon dioxide, ethanol, lactic acid, formic acid, etc. (John et al. 2011). A patented process is based on this sort of fermentation (Ueda et al. 1996), in which microalgal cells contained a large amount of polysaccharides, which were catabolized under dark and anaerobic conditions to ethanol. This process does not apply to all species of microalgae, but according to (Ueda et al. 1996), classes *Chlorophyceae*, *Prasinophyceae*, *Cryptophyceae*, and *Cyanophyceae* are the ones able to be induced to produce ethanol.

As well explained by John et al. (2011), the algal photosynthesis is based on Calvin cycle in which ribulose-1,5-bisphosphate (RuBO) combines with CO_2 to produce two 3-phosphoglyceric acid (3-PGA), which is used to produce glucose and other several metabolites. There is a current attempt trying to redirect the 3-PGA produced to ethanol transformation. This is mainly done by introducing ethanol producing genes, such as pyruvate decarboxylase and alcohol dehydrogenase (John et al. 2011). Deng and Coleman (1999) published a work using modified cyanobacterium (*Synechococcus* sp.) in order to utilize light, CO_2 and inorganic nutrients to produce ethanol and have it diffused from the cell into the culture medium.

Ethanol producing from microalgae is, thus, a challenge for biotech companies. There are a few bottlenecks in these three processes; such as the high cost of starch/cellulose depolymerizing enzymes for pretreatment of algal biomass and the competition with higher value fuels.

17.3.4 Biohydrogen from Microalgal Biomass

Microalgae generally have the necessary genetic, metabolic, and enzymatic characteristics to photoproduce H_2 gas. There are two possible pathways for hydrogen production under anaerobic conditions from eukaryotic microalgae: either as an electron donor in the process of fixating CO_2 or evolved in both light and dark (Ghirardi et al. 2000; Melis and Happe 2001).

During the process of photosynthesis, microalgae convert water into H^+ and oxygen. H^+ can be subsequently converted to H_2 through hydrogenase catalyzed reactions under anaerobic conditions (Melis and Happe 2001; Cantrell et al. 2008). The key for producing hydrogen in this situation is the utilization of anaerobic environments, since oxygen is a key inhibitor to hydrogenases (Akkerman et al. 2002). This reaction is reversible, therefore, hydrogen is either produced or consumed by the conversion of protons into hydrogen gas.

Brennan and Owende (2010) cited two fundamental approaches for photosynthetic H_2 production from water. The first one is a two-stage photosynthesis process in which photosynthetic oxygen production and generation of hydrogen gas are spatially separated. The first stage of this process consists of microalgae growing in normal conditions; the second one consists of privation of sulfur, which induces anaerobic conditions, stimulating hydrogen production (Melis and Happe 2001). This is a time-limited process, and hydrogen yields achieve a maximum after 60 h of production.

The second approach consists of simultaneously producing oxygen and hydrogen gases. In this process, the hydrogenase reaction is fed directly with electrons that are released upon oxidation of water (Ghirardi et al. 2000). There is a considerable higher productivity in this process, when compared to the first, however, hydrogenases are inhibited after a short time by the oxygen produced. Melis and Happe (2001) calculated the theoretical maximum yield of hydrogen using the two-step process and found numbers as high as 198 kg H_2 ha^{-1} day^{-1}.

There is little research yet on biohydrogen production from microalgal derived routes. The main challenge is to achieve high yields, in order to make this process feasible, since the theoretical photochemical efficiency of the photoheterotrophic process is low, of around 10 %. Even achieving high yields, with very high light intensities, still a large surface would be needed to reach a reasonable hydrogen production (Melis and Happe 2001). A summary provided by Melis and Happe (2001) is shown, comparing efficiencies in Photossynthetically active radiation (PAR) and H_2 production, in Table 17.4.

17.3.5 Pyrolysis of Microalgal Biomass

Microalgal biomass can be converted to bio-oil, syngas, and charcoal through pyrolysis. Pyrolysis processes happen at medium range temperatures (350–700 °C) in the absence of air (Goyal et al. 2008). There are a few operating modes of pyrolysis, as well described by Bridgwater (2012), which are called: Flash, Fast, and Slow pyrolysis.

Flash pyrolysis works at moderate temperatures of 500 °C, it has a short hot vapor residence time, of about 1 s, and is deemed to be a viable technique for future replacement of fossil fuels with biomass derived liquid fuels, since there is a high biomass-to-liquid conversion ratio. This ratio achieves numbers as high as 95.5 % (Clark and Deswarte 2011; Demirbaş 2006). Fast pyrolysis also works at

Table 17.4 Energy conversion efficiencies of green algae for H_2 production

Species	Absorbed light ($\mu W/cm^2$)	H_2 (nmol h^{-1})	Efficiency (PAR) %	References
C. reinhardtii (sup)	2.2	44–61	13–18	Melis and Happe 2001
C. reinhardtii (UTEX 90)	8.4	78–104	6–8	Melis and Happe 2001
Chlorella moewusii	9.1	253–337	18–24	Melis and Happe 2001

The results are based on several different light periods and with a heating value of H_2 of 0.23 MJ/mol

the vapor residence time, of around 10–20 s. Slow pyrolysis, on the other hand, is processed at lower temperatures, of around 400 °C and has very long solids residence time. The liquid percentage in these three processes are, respectively, 75, 50, 30 %; the char is known to be around 2, 20, and 35 % and the gas percentage of 13, 30, and 35 % for flash, fast, and slow pyrolysis, respectively. These numbers are based on Bridgwater (2012).

Bio-oil from microalgal biomass has higher quality than the one extracted from lignocellulosic materials (Demirbaş 2006), making it a promising area of studies. Bio-oils have been preferred over the other products of pyrolysis because they have the potential for being upgraded to liquid transportation fuels (Chen et al. 2010).

Pyrolysis of microalgal biomass converts lipids, starch, protein, and cellulose into bio-oil, combustible gas, and charcoal (Chen et al. 2010; Ginzburg 1993). It is interesting to note that the products from heterotrophic and from autotrophic grown microalgae can be very different; these effects are believed to be due to different methabolic pathways during their growth (Miao and Wu 2004).

Some current challenges in making pyrolysis from microalgal biomass feasible, presented by Chen et al. (2010), are: the dewatering process prior to the pyrolysis itself which is a very high energy requiring step, and the fractioning of the resulting bio-oil. Bio-oil can achieve high levels of component complexity and acidity as well. There is a field of studies in testing different conditions and catalysts to improve bio-oil quality (Wan et al. 2009).

A new approach in microalgal biomass pyrolysis is the utilization of microwaves. This technology, developed at the University of Minnesota, provides a few important advantages toward the conventional processes (Du et al. 2011), such as easier to control heating, fewer requirements on the feedstock grinding, cleaner conversion products, produced syngas with a higher heating value, and low cost.

17.3.6 Other Energy Products from Microalgal Biomass

Algal biomass can also be converted to a combustible gas mixture called "synthesis gas," or simply syngas. These reactions consist of partial oxidation of biomass in the range of temperatures from 700 to 1100 °C (Chen et al. 2010). As

well as pyrolysis, gasification products vary according to the temperature, moisture content, and other factors. The major applications of syngas are based as source of thermal energy in gas engines or gas turbines and feedstock for catalytic reforming and fermentation, in order to produce other chemicals.

Gasification of microalgal biomass has had little interest over the past years, thus making a broad field of study available for researchers and research groups with available technology to work on this. Demirbas (2009) aimed at producing H_2 from microalgal biomass, having CO_2, CO, and CH_4 as byproducts through a gasification process. Although there are some companies working on syngas production, such as Ensyn Corp and Plascoenergy Group, both Canadian industries, few research has been developed using microalgal biomass. Gasification and catalytic reforming of residue biomass might be an answer for achieving higher sustainability index from microalgal derived fuels. A new approach is to include algal charcoal, after biomass utilization, as a new product in the portfolio of algal products (Johnson et al. 2013).

There is also the energetic route known as thermochemical liquefaction, which aims to produce liquid fuel from wet algal biomass (Patil et al. 2008). The so-called bio-oil derived from liquefaction is produced at a range of low temperatures, usually from 300 to 350 °C, at high pressures, from 5 to 20 MPa, with the presence of a catalyst and in the presence of hydrogen. The mechanism of bio-oil production through this process is the high water activity in subcritical thermodynamic conditions in order to decompose, i.e., break down biomass to smaller molecules with a higher energy density (Patil et al. 2008). Dote et al. (1994) produced bio-oil with a heating value of 45.9 MJ kg^{-1}, with an yield of 64 % at dry weight of biomass and a positive energy balance of 6.67:1 (output/input). These numbers, especially the last one, may make thermochemical liquefaction of algal biomass a promising alternative for further energetic studies.

Hydrothermal processes have several technical and engineering challenges, such as controlling the ideal heating rate, the residence time, and up-scaling processes. Most studied processes are still in batch conditions, which may show a wide range of temperatures and a long time to have the reactor cooled down before analysis of products. Continuous processes would theoretically show better results, since these problems are minimized (Chen et al. 2010). Therefore, some of the engineering challenges would be improving heating rate, making a homogeneous flow (in order to prevent clogging) and higher-pressure pumps.

A relatively less promising option, which could be used as one of the last steps in the algal biomass lifecycle, is the direct combustion of biomass. Combustion is the oxidation reaction at high rates in presence of air and high temperatures, of 800 °C or more, at furnaces, boilers, or steam turbines. Not only algal, but any biomass should have a maximum moisture content of 50 % (McKendry 2002). A drawback for growing algal biomass solely for combustion is the need for drying, chopping, and grinding, which raises costs, producing a relatively cheap product. Therefore, it may not be feasible to burn directly biomass without extracting higher value products, such as lipids and proteins.

17.4 Challenges in Optimizing Sustainability

Prior to any discussions regarding biomass purification and extraction of products, it is important to discuss what would be the best way to cultivate microalgae. As previously shown in this chapter, the utilization of photobioreactors may yield higher productivity yields; on the other hand, it shows higher costs than other cultivation methods. The cheapest way to produce microalgae in large scale would be utilizing lagoons, lakes, or open ponds. In these, however, without any light administration, there would be a daily loss of biomass of around 25 % due to overnight respiration (Ratledge and Cohen 2008), not even mentioning the possibility of contamination from protozoa, bacteria, other algae, and fungi. Yields in lagoon systems would require up to 2 months for the culture to reach an optimum biomass density, in order to harvest.

As previously described, collecting and concentration algal biomass are cost intensive processes and have been subject of study in order to enhance sustainability from algal biofuels. A study from the 1960s was made comparing several harvesting techniques, including filtration, flocculation, precipitation, ion exchange, and ultrasonic vibration (Golueke and Oswald 1965). The authors concluded that only centrifugation and chemical flocculation were economically viable at the time. With environmental concerns nowadays, chemical flocculation may have some drawbacks, since the usage of chemicals in these processes is not incentivized; centrifugation may not be the most feasible option as well, because it is an energy intensive process and may damage cell wall, which can represent yield loss in some extraction steps. Alternatives, such as the usage of low energy ultrasound waves, are gaining room in microalgae production. These sort of waves allow cell to aggregate and settle down once the ultrasonic field is turned off. The main disadvantage, common among some new techniques, is the high power consumption and low concentration factors (Bosma et al. 2003).

Up to the date, it is proven that oil extraction from microalgae is an expensive and difficult process. There is no well-defined and ready to scale-up method available on the market and most of extractions face challenges with chemical waste and/or high costs of operation. There are some new technologies, such as nano-dispersion (promotes dispersion of nano-sized particles), electroporation, and the usage of co-solvent systems (Chen et al. 2010). Once again, engineering challenges are faced in the oil extraction step, and whichever method makes itself more sustainable and economically feasible will definitely attain market interest. Dewatering is also another key issue previously discussed. Thus, an extensive engineering work must be done in order to make oil production feasible and reduce the minimum cost of today production of US$5600–7000/ton (Ratledge and Cohen 2008).

Although with all the required processing steps in order to achieve the oil extraction step, its characteristics may limit biodiesel production. First of all, even though some microalgae species are able to accumulate up to 70 wt% of lipids

(*Botryococcus braunii*) (Chen et al. 2010) under starvation of N, P, and Si, the overall yield may not be high enough to make it economically feasible. The development of genomic engineering to map all the pathways in the algal cell, especially using *Chlamydomonas reinharttii*, has been done in order to fully understand lipids production and, obviously, address its optimization afterwards.

Algal oil characteristics are also challenges toward commercialization of fungal biodiesel, for example. The high free fatty acid value and the presence of unsaturated bonds are two drawbacks in the biodiesel industry, since series of pretreatments, higher costs with catalysts (since the cheapest and most traditional in biodiesel plants, NaOH and KOH, cannot be used), and lack of oxidation stability are faced.

In the 1960s, Japan started to produce *Chlorella* as a food additive, and since then, the potential of using microalgae in the food industry has grown enormously. Today, the most used species in human nutrition are primarily from *Chlorella*, *Spirulina*, and *Dunaliella* classes (Brennan and Owende 2010). The high content of beta-carotene in *D. salina* (up to 14 %) (Moore 2008) and the usage of *Chlorella* sp. in the pharmaceutical industry make the biofuels industry less advantageous, comparing economically values. Going further, microalgae can also be source of high value PUFA, such as docosahexaenoic acid (*Crypthecodinium* and *Schizochytrium* spp.), eicosapentanenoic acid (*Nannochloropsis*, *Phaedactylum*, *Nitzchia*, and *Pavlova* spp.), γ-linolenic acid (*Spirulina* sp.), and arachidonic acid (*Porphyridium* sp.) (Spolaore et al. 2006). In addition, microalgae can be source for pigments, aquaculture feed, high value fertilizer, and biochemical isotope chemicals, that have higher value than biofuels (Spolaore et al. 2006). The challenge of facing these industries could be deviated using a combined platform, producing these higher value products and also further processing algal biomass, producing biodiesel, methane, bio-oil, etc.

17.5 Opportunities and Other Applications of Microalgae

17.5.1 Growth in Municipal Leachate

There are a few publications about the usage of microalgae toward landfill leachate purification. They could utilize organic compounds present in there as carbon and nitrogen sources (Lin et al. 2007; Cheung et al. 1993). A recent project at the University of São Paulo, Brazil, aims at the utilization of leachate as culture media for *Chlorella* sp. growth. The current stage of this project is the chemical, physical, and nutritional factor screening toward cell growth.

Table 17.5 Reported values of heavy metals uptake by *C. vulgaris*

Metal	mg g^{-1} (metal/biomass)	References
Au	25.02	Ting et al. (1995)
Cd	12.48	Sandau et al. (1996)
Cu	190.62	Mehta and Gaur (2001)
Ni	205.48	Mehta and Gaur (2001)
Pb	17.2	Sandau et al. (1996)
Zn	6.6	Sandau et al. (1996)

17.5.2 Cocultivation with Pelletized Fungus

A recent study at the University of Minnesota (Hu et al. 2013) is based on a novel approach of utilizing microalgae and pelletized fungus for a series of advantages. The coculture enables filamentous fungi, under pelletized morphology, to have microalgae attached on the pellets; which may drastically decrease harvesting costs, avoid second pollution from flocculants and the researchers claim that it also stimulates the algae production (Zhang and Hu 2012).

The fungal pellets (*Aspergilus niger*), with an average diameter of 2–5 mm, act as nuclei for microalgal cells to attach. The proposed mechanism for this phenomenon is due to the production of hydrophobins, which are hydrophobic proteins, a family of low molecular weight amphipathic proteins (Linder 2009) detected hydrophobin on the fungal hyphae, and one of the functions of these proteins is to coordinate the adherence of hyphae to solid substrates. This study still needs inputs for larger scale purposes, and may be one of the answers for enhancing higher sustainability from microalgae-derived fuels.

17.5.3 Metal Sorption

The so-called biosorption is the capability of passive removal of toxic heavy metals such as Cd^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, Cr^{3+}, and Hg^{2+} by inexpensive biomaterials (Davis et al. 2003). Some green algae, such as *Chlorella* spp., *Cladophora* spp., *Scenedesmus* spp., *Chlamydomonas reinhardttii,* have been studied due to their capability of some metals sorption, and the affinity of sorbing a particular ion is particular to a species and to the physical conditions the cells are grown, since the cell wall composition may change (Mehta and Gaur 2005).

Chlorella vulgaris, for instance, has a broad spectrum of metal sorption, such as those reported in Table 17.5. These numbers are based on (Mehta and Gaur 2001; Sandau et al. 1996; Ting et al. 1995).

17.5.4 Microalgal Biorefinery

The concept of a microalgal-based refinery is based on a traditional petroleum refinery. Since many products are possible to be produced from microalgal biomass, as well as their utilization as cleaning and depolluting agents, microalgae would be able to be source of this type of industry. If adequate project and systems studies are applied, one can achieve maximization on revenues from these organisms, achieving high economic and environmental benefits.

Unlikely a petroleum refinery, a biorefinery utilizes biomass as feedstock for its operation, producing a wide range of products from one or more biological resources (Chen et al. 2010). An integration approach can be applied, making it possible to produce multiple products from a single biomass feedstock, and this system can also be self-sufficient in energy, in case of using biomass residues as energy source.

17.6 Conclusion

Although microalgae have been an interesting field of study in the most diverse areas of engineering, microbiology, and biochemistry, it still needs further technical development to make an algal biorefinery something feasible, making products sufficiently cheap, sustainable, and profitable. As argued by Ratledge (2008), oil contents of algal cells should be at least 40 % or above to be a starting material for biodiesel, for example. According to them, producing methane through anaerobic digestion would yield very little revenue, as well as burning the residual biomass.

Chen et al. (2010) cited a few key economic concerns of the mass algal production systems; which are basically: the cost of the resources for producing microalgae, the cost of construction and maintenances of the culture system, the operational costs of harvesting systems, downstream processing and refining. It is clear that costs vary according to location, solar energy availability, species, etc.

Microalgae does not show any direct competition with the food supply system, which is an attractive point toward its production. However, the well-established method for producing *Spirulina* for consumption is very simple (usage of lakes and natural lagoons, without mechanical stirring and simple methods of harvesting and sun drying) and produces a higher value products than, for instance, fuels. Its biomass is also source of beta-carotene and PUFA, which have a great interest from food and pharmaceutical industries.

A very favorable point when growing microalgae is the carbon capture issue. Also according to Chen et al. (2010), for every ton of algal biomass produced, approximately one ton of carbon dioxide is fixed (assuming 40 wt% of dry algal biomass as carbon). While most plants capture very dilute CO_2 from the

atmosphere, most algae are able to use very concentrated CO_2 as carbon source, allowing it to be part of industrial effluent cleaning processes for example.

Therefore, it is clear that an extensive work must yet be done in order to make microalgae a major source for energy production a feasible option. The dream of making a microalgae-derived refinery is far in the near future reality, but it shows potential of becoming an alternative of supplementing and even replacing non-renewable fuels. The possibilities of growing microalgae under autotrophic conditions and of utilizing its properties to clean air and wastewater are some unique advantages that are counting toward its feasibility. Economic studies have shown also that commodity oils, such as soybean, have doubled and even trebled within one year (Ratledge and Cohen 2008). Following this trend, there will be an equivalent point in which commodity oil and algal oil will match in price and from this point onward, algal oil should be cheaper. Within 10–20 years, there should be innumerable research advances which will probably derive microalgae as a potential energy source in the world.

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