Chapter 17
Green Microalgae as Substrate for Producing Biofuels and Chlorophyll in Biorefineries

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Abstract In order to develop sustainable technologies with reduced impacts in an attempt to repair previously caused damages to the environment, green alternatives have been the focus of several researches nowadays. Technological advances have enabled an effective use of natural sources to obtain clean energy, thus reducing emissions of gaseous pollutants into the environment. In this context, biofuels are promising alternatives for regulating climate change caused by an increase in the greenhouse effect, whose negative impact has been considerably perceived over the years. The use of microalgae as raw material to obtain biofuels has been proved promising. Due to the rich composition of carbohydrates, lipids and various proteins, biofuels and bioproducts can be obtained from microalgal cells, thereby contributing to bring down the final cost of products within the concept of biorefineries. Thus, this chapter aims to identify the process variables that interfere in microalgae cultivation to produce biofuels and pigments, and their impact on microalgal cell composition. Information on the most widely used culture media and the most studied species for obtaining biofuels by focusing on biohydrogen, biodiesel and bioethanol have been assessed. Furthermore, the process for obtaining these biofuels was illustrated in a simplified form in order to provide a general overview for readers.

Keywords Microalgae cultivation · Third generation biofuel · Renewable energy

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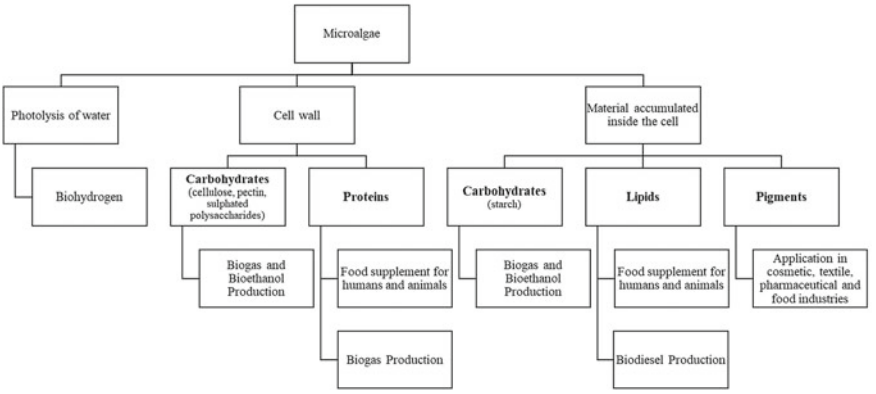

Fig. 17.1 Biotechnological possibilities of microalgal biomass utilization within the concept of biorefineries

17.1 Introduction

Microalgae are unicellular, microscopic and photosynthetic organisms found in both freshwater and seawater (Velasquez-Orta et al. 2013). They can also proliferate in industrial and urban wastewaters (Caporgno et al. 2015; Lu et al. 2015; Wu et al. 2017), and icy waters of oceans (Begum et al. 2016). These organisms are part of phytoplankton and depend essentially on sunlight, carbon dioxide (CO_2) and water for producing structural molecules, reserve molecules and pigments, consequently leading to cell proliferation (Koller et al. 2014). According to Spolaore et al. (2006) and Koller et al. (2014), microalgae and their extractives have been widely used in both human and animal nutrition, and as raw materials to be used in textile, pharmaceutical, cosmetic and food industries. In addition, they can be used as feedstock for obtaining biofuels, as shown in Fig. 17.1.

17.2 Microalgae Cultivation to Produce Biofuels

Since the beginning of the industrial revolution, technological development has been based on the combustion of fossil fuels derived from petroleum, natural gas and coal. These energy sources are not renewable and their burning promotes the emission of gases that lead to the greenhouse effect, such as CO_2. According to Alley et al. (2007) and "Global Climate Change" (2015), CO_2 atmospheric concentration has increased from 280 ppm in the year 1750 to 400.47 ppm in 2015. This 43% increase disrupted the balance between the solar radiation that is received and reflected by the Earth, thus increasing its ability to retain heat and resulting in the so-called global warming phenomenon (Höök and Tang 2013; Peters et al. 2013; Scheutz et al. 2009). This

scenario becomes extremely alarming by considering the drastic climate change that has been observed over the years, which has been clearly intensified recently.

The search for sustainable alternatives aimed at preserving the environment and controlling the increased emission of greenhouse gases (Samimi and Zarinabadi 2012), as well as a constant rise in the price of petroleum (Nazlioglu and Soytas 2012) has encouraged research in the area of bioenergy. Thus, energy resources capable of being naturally renewed at a fast pace and that are directly and indirectly obtained from energy sources such as the sun, wind, waves, plant and microalgal biomass have been explored (Jacobson and Delucchi 2011; Long et al. 2013). In this sense, three generations of biofuels, first (1G), second (2G) and third (3G) generations, can be obtained.

Both 1G and 2G biofuels are obtained from plant biomass. 1G biofuels are produced from sugar fermentation for ethanol generation, which can be obtained mainly from corn, beet, wheat and sugarcane juice, or by transesterification of lipids to be used in the food industry, especially those of vegetable origin, e.g. soybean oil, for biodiesel production. On the other hand, 2G biofuels are mainly obtained by using sugars extracted from agro-industrial waste, such as straw and bagasse, or lipids from non-food sources, e.g. jatropha oil (Naik et al. 2010). Although the production of these biofuels is classified as a sustainable process and is among the green technologies which are so encouraged as a way of preserving the environment, one of the major drawbacks of this process is the need for arable or pre-treated soil to obtain sugars, due to the recalcitrance of vegetable raw material.

3G biofuels are an alternative to 1G and 2G and, instead of being obtained from plant biomass, are the result of processing microalgae cells. The advantages of cultivation and obtainment of biofuels from microalgae in comparison with using vegetal biomass are listed in Table 17.1 (Chen et al. 2014).

Due to the rich composition of lipids and carbohydrates and the ability to fixate CO_2, (Sankar et al. 2011) several types of biofuels can be produced from microalgae (Fig. 17.1). The oil accumulated inside the cell can be extracted and used for biodiesel production (Gong and Jiang 2011). After lipid extraction, the residual biomass can be used for biogas or bioethanol production. Biogas production is carried out from an anaerobic digestion of microalgal biomass (Mussgnug et al. 2010), while bioethanol production is performed through the hydrolysis of sugars present in the cell wall and fermentation by microorganisms (Hernández et al. 2015). The stages of oil extraction and microalgal biomass processing to obtain ethanol are depicted in Fig. 17.2. The biohydrogen produced by photolysis at the photochemical stage during photosynthesis can be stored (Batyrova et al. 2015; Benemann 2000). However, studies on biofuel co-generation with the same culture have been carried out, as shown in Table 17.2.

Table 17.1 Advantages and disadvantage of using microalgae to obtain biofuels

Advantages	Disadvantage
• Microalgal cells can be cultured in small spaces • Microalgae proliferation rate of is higher than plant growth time • The absence of structures such as stem and leaves, together with the fact that they are submerged in water, make them more efficient in the conversion of solar energy into biomass when compared to superior plants • High accumulation rate of lipids, carbohydrates and proteins • Harvesting cells can be performed anywhere • They can be grown in non-potable water (wastewater) • Other products of high commercial value, in addition to biofuels, can be obtained • They are adaptable to various geographic or climate conditions	• Need for large amounts of cells to obtain a satisfactory quantity of extractives

Table 17.2 Biofuel cogeneration from the same microalgae culture

Microalgae	Biofuel	References
Chlamydomonas reinhardtii	Biohydrogen Biogas	Mussgnug et al. (2010)
Chlorella sp.	Biohydrogen Biodiesel	Dasgupta et al. (2015)
Co-culture of *Scenedesmus* sp. and anaerobic sludge in starch-rich wastewater	Biohydrogen Biodiesel	Ren et al. (2015)
Chlorella sp.	Biohydrogen Biodiesel	Sengmee et al. (2017)
Chlorococum sp.	Biohydrogen Bioethanol	Harun et al. (2010)

17.2.1 Process Variables

Microalgal cell composition may vary according to species, culture and/or environmental conditions (Brown 1991; Rhee 1978; Volkman et al. 1989). Other factors such as variation in temperature (Renaud et al. 2002), supplementation (Jiang et al. 2012; Procházková et al. 2014; White et al. 2013), luminous intensity and photoperiod (Khoeyi et al. 2012) may significantly alter microalgal cell composition. Under optimal culture conditions, microalgae multiply rapidly but do not accumulate reserve substances (carbohydrates and lipids). On the other hand, adverse conditions tend

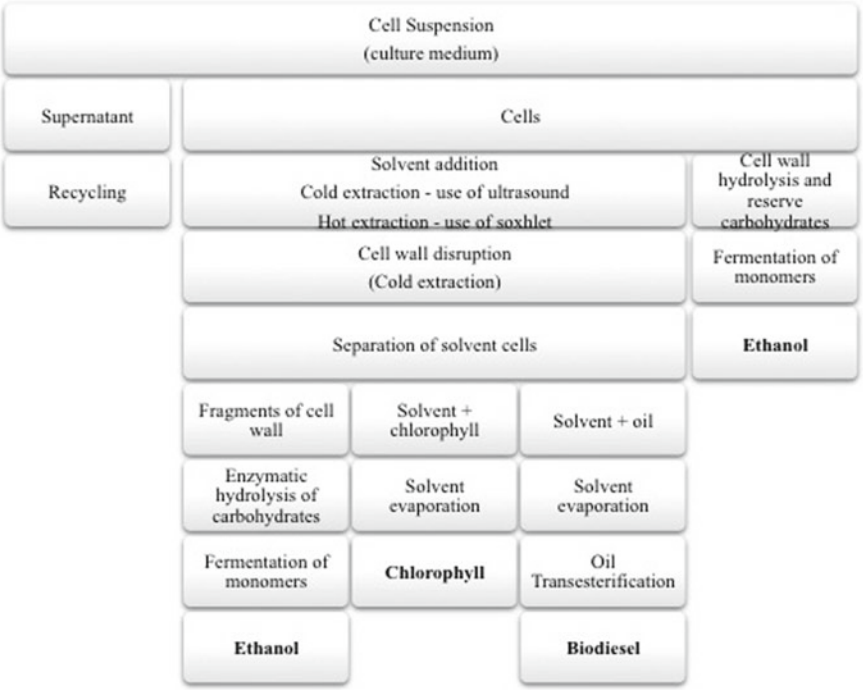

Fig. 17.2 Stages to obtain bioethanol, biodiesel and chlorophyll from green microalgae cultivation

to stimulate the accumulation of reserve substances or pigments. Thus, products or by-products of interest can be obtained by regulating experimental conditions.

Microalgae can be cultured either in the presence or absence of light, and can use both organic and inorganic carbon as an energy source. Thus, cultures can be performed in four different conditions: (i) photoautotrophic, (ii) heterotrophic, (iii) photoheterotrophic and (iv) mixotrophic (Fig. 17.3).

Photoautotrophic condition is the most used and is performed in the presence of a light source, which enables the conversion of inorganic carbon into energy through photosynthesis process. Culture illumination can be carried out by means of a natural light source (solar energy) or by light bulbs. It is emphasized that the intensity and color of light and the emitted wavelength directly interfere in the development and accumulation of biomolecules by cells (Kim et al. 2013; Zhao et al. 2013). Some microalgae are able to use organic carbon as a source of energy and carbon, thus characterizing heterotrophic culture. In such a case, light energy is not required for biochemical reactions to occur. In mixotrophic and photoheterotrophic conditions, cultures are carried out in the presence of light and an organic carbon source. The difference between these conditions is that, during mixotrophic culture, cells perform photosynthesis and use both organic and inorganic carbon to obtain energy, i.e. they are able to live in both photoautotrophic and heterotrophic conditions. On the other

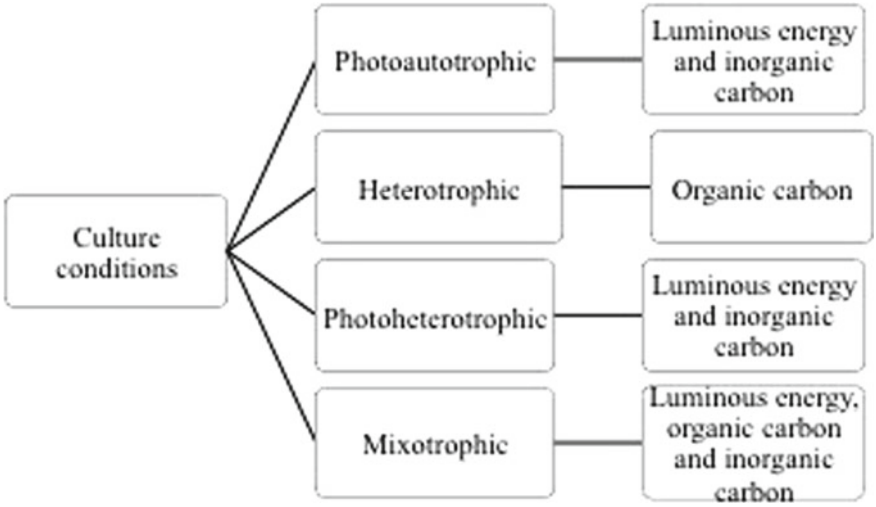

Fig. 17.3 Types of condition used for microalgae cultivation

hand, in a photoheterotrophic condition, cells need light to use organic compounds as carbon source (Chen et al. 2011). These conditions are used to study the production of hydrogen (Table 17.2), accumulation of lipids (Table 17.3) and carbohydrates (Table 17.4) by microalgal cells to evaluate biohydrogen, biodiesel and bioethanol yields, respectively.

Although the production of large quantities of microalgal biomass is required to commercially produce biofuels, most studies report cultures in laboratory scale using artificial lighting (indoor), usually in photobioreactors with 1 to 10 L of capacity. On the other hand, scale-up (Oncel et al. 2015) and pilot-scale (Chen et al. 2014; Lu et al. 2015) simulation studies using outdoor lighting have been conducted to increase productivity (Tables 17.2, 17.3 and 17.4).

Culture medium composition is of major importance for cell growth and proliferation. Different species of microalgae present different nutritional requirements, although they can adapt to different supplementation conditions. For this reason, industrial (Abdel-Raouf et al. 2012; Lu et al. 2015; Wu et al. 2017; Yu et al. 2014) or domestic (Lv et al. 2017; Reyimu and Ozçimen 2017) wastewaters which are either concentrated or diluted can be used as culture medium. However, culture media with previously established composition (synthetic) may have added or subtracted nutrients, or the concentration of one or more components may be altered in order to evaluate the response of cells to this new condition. Therefore, supplementation should be adequate to the biochemical route that is to be stimulated in order to obtain the product of interest. The use of low cost nutrient sources such as glycerol/glycerin (Li et al. 2011; Sengmee et al. 2017), human urine (Wu et al. 2017) and urea (Campos et al. 2014) has been explored. Examples of culture media used for producing H_2, lipids and carbohydrates are listed in Tables 17.2, 17.3 and 17.4,

Table 17.3 Culture media and types of photobioreactors for H_2 production from different microalga species

Microalgae	Culture media	Reactor	Agitation	Culture condition	Culture type	Hydrogen production	References
C. reinhardtii	TAP	Cylindrical reactor vessels (600 mL)	Mechanical and magnetic stirring	PA	Indoor	H_2 production begins after anaerobiosis in the culture system	Antal et al. (2003)
C. reinhardtii	High salt content media	Glass PBR with pH and O_2 sensors	Magnetic stirring	PH	Indoor	56.40 mL/L	Tsygankov et al. (2006)
C. reinhardtii CC124	TAP	Tubular PBR (110 L)	Bubbling of a mixture of air and CO_2	PA	Indoor	Productivity: 0,61 mL of H_2/L/h	Giannelli and Torzillo (2012)
C. reinhardtii Dangeard 137C mt+	TAP	Microprocessor-controlled PBR	Magnetic stirring	PA	Indoor	109.00 mL/L	Batyrova et al. (2012)
C. reinhardtii CC124 mutante	TAP	Flat plate Roux type PBR (H_2 production) (800 mL)	Mechanical stirring	MT	Indoor: simulação de condição outdoor	Productivity: 1.30 mL of H_2/L/h	Oncel et al. (2015)
Chlorella sp.	TAP glicerol	Serum bottle	Ns	MT	indoor	Maximum production: 10.31 ± 0.05 mL/L	Sengmee et al. (2017)
		Not specified (1 L)				Maximum production: 11.65 ± 0.65 mL/L	
Scenedesmus sp. NBRI012	TAP no sulphur	Rectangular glass reactor (2 L)	Magnetic stirring	PA	Indoor	17.72% $v_{H2}/v_{total\ de\ gases}$	Dasgupta et al (2015)

PBR photobiorreator; *TAP* Tris-acetate-fosfate; *PA* photoautotrophic; *PH* photoheterotrophic; *MT* mixotrophic; *Ns* not specified

Table 17.4 Culture media, types of photobioreactors and cultures to obtain oil from different species of microalgae

Microalgae	Culture media	Reactor	Culture condition	Culture type	Agitation	Composition/productivity	References
Chlorella vulgaris	Fitzgerald modified	PBR (5 L)	PA	Indoor	Aeration (air and CO_2)	Composition: 29.53% of total lipids in dry biomass Productivity: 12.77 mg lipid/L/d	Widjaja et al. (2009)
Chlorella minutissima UTEX2341	Basic N8Y	*Erlenmeyer* flasks (500 mL)	PH	Indoor	Shaking	Composition: 16.11% lipid esterifiable in dry biomass with 62.97% FAME conversion Productivity: 286.76 mg lipid/L/d and 180.68 mg FAME/L/d	Li et al. (2011)
Chlorella sp.	Bold Basal	PBR (10 L)	PA	Indoor	Aeration (air)	Composition: 12% total lipids in dry biomass, of which 42% were esterifiable	Velasquez-Orta et al. (2013)
Nannochloropsis oculata						Composition: 24% total lipids in dry biomass, of which 17% were esterifiable	
C. vulgaris ESP-31	Basal media and Bristol modified	Vertical tubular-type (50 L)	PA/PH	Outdoor	Aeration (air and CO_2)	Productivity: 48 mg lipid L/d	Chen et al. (2014)
C. vulgaris (FWM-CV)	MBL adapted to fresh water	*Erlenmeyer* flasks (5 L)	PA	Indoor	Aeration (air)	Composition: 19.27% of total lipids in dry biomass Productivity: 2.19 mg lipid/L/d	Al-Lwayzy et al. (2014)
Chlorella kessleri UTEX2229	Domestic wastewater	Flat-panel airlift (1 L)	PH	Indoor	Aeration (air and CO_2)	Composition: 7.4% of esterifiable lipids in dry biomass	Caporgno et al. (2015)
C. vulgaris CCAP211/19						Composition: 11.3% of dry biomass esterifiable lipids	
Chlorella sp.	Dairy industrial wastewater	PBR (1, 3 L)	PA	Indoor	Aeration (air)	Composition: 55.54 mg of fatty acid methyl esters/g dry weight	Lu et al. (2015)
		PBR (30 L)		Outdoor		Composition: 34.90 mg of methyl esters of fatty acid/g dry weight	

(continued)

Table 17.4 (continued)

Microalgae	Culture media	Reactor	Culture condition	Culture type	Agitation	Composition/productivity	References
Scenedesmus sp. NBRI012	TAP (no S)	*Erlenmeyer* flasks (1 L)	PH	Indoor	Magnetic stirring	410.03 mg of lipids/L	Dasgupta et al. (2015)
Chlorella sp. NBRI029						587.38 mg of lipids/L	
C. minutissima	Tamiya	tubular PBR (120 L)	MT	Indoor	Aeration (air and CO_2)	45.82% of polyunsaturated fatty acids	Aremu et al. (2015)
			PA			37.90% of polyunsaturated fatty acids	
C. minutissima UTEX2341	Artificial wastewater	*Erlenmeyer* flasks (500 mL)	PH	Indoor	Orbital shaker	Productivity: 249.36 mg of lipids/L/d in medium containing 4 mM cadmium	Yang et al. (2015)

PBR photobiorreator; *PA* photoautotrophic; *PH* photoheterotrophic*; MT* mixotrophic*; CO_2 gás carbônico; S enxofre; N nitrogênio*

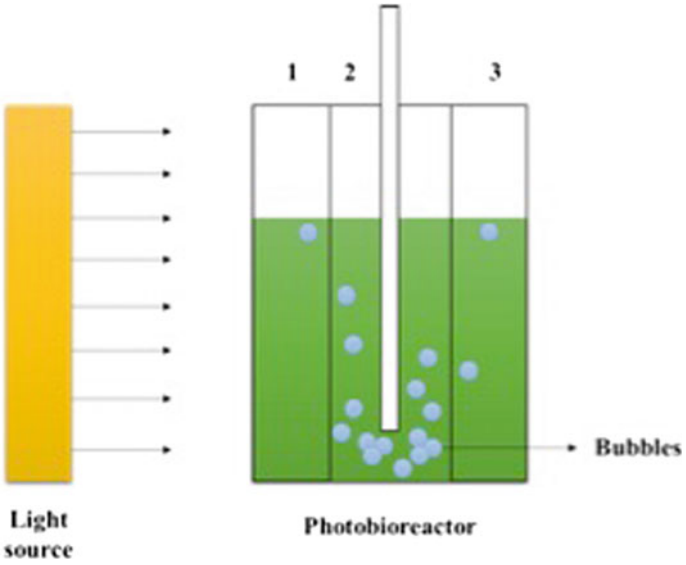

Fig. 17.4 Schematic of a bench photobioreactor with agitation by air bubbling and identification of illumination zone (1), intermediate zone (2) and dark zone (3)

respectively. It should be emphasized that the culture medium should be adequate to each species needs, so that marine microalgae can be cultivated in a high osmotic pressure environment in order to resemble seawater characteristics. In such a case, high concentrations of sodium chloride (approximately 30 g/L) are used.

Stirring in microalga cultures is essential to prevent cell sedimentation and ensure cell suspension homogenization. As regards a bench photobioreactor (PBR), stirring is essential to ensure that all cells receive the same amount of light in photoautotrophic and photoheterotrophic (indoor) cultures. In this type of culture, the light source is usually placed specifically towards the PBR (Fig. 17.4) and cells located in the illumination zone block the passage of light into the dark zone as cell proliferation progresses. One way to avoid shading between cells is by installing lighting sources on opposite sides of the PBR or wrapping it with light strips.

Flow can be produced by mechanical agitation, air and/or gas bubbling or with the aid of a peristaltic pump. Although mechanical agitation is more efficient when compared to other types of agitation, contact with the agitator can damage the cells. Another downside is the elevated cost involved in installing the agitator and operating the system, thus discouraging its use on an industrial scale. In addition to promoting lower cell damage rate, the bubbling or aeration system requires less financial investment and is easy to be installed when compared to mechanical agitators (Chisti 2008). Nevertheless, this system has the advantage of, in addition to conserving suspended cells, the culture medium being aerated. Examples of agitation types, reactor types and cultivation conditions can be seen in Tables 17.2, 17.3 and 17.4.

17.2.2 Hydrogen Production by Microalgae

According to Momirlan and Veziroglu (2002), H_2 is the most promising fuel for replacing fossil fuels in the medium and long term when compared to other known fuels, due to its high energy density per mass. In addition to being a renewable energy resource, it is considered an ideal alternative to fossil fuels since it does not increase the greenhouse effect. This is due to the fact that, upon combustion, H_2 produces only water and can be used for power generation by fuel cells, or directly in internal combustion engines. However, H_2 use as fuel is considered limited due to high production costs, and transportation and storage difficulties (Khetkorn et al. 2017).

H_2 can be produced from fossil fuels (Steinberg 1989), natural gas (Block et al. 1997) or water. H_2 production from water can be accomplished by numerous processes, including liquid water or steam electrolysis (Zeng and Zhang 2010), photolysis (Barrett and Baxendale 1960), thermochemical decomposition (Funk 2001) and photoelectrochemical process (Sivula et al. 2010). Biological H_2 production is performed by microorganisms through photosynthetic or non-photosynthetic processes (Khetkorn et al. 2017). Non-photosynthetic H_2 production can be performed under aerobic conditions using inorganic carbon, such as CO_2, or through anaerobiosis using an organic carbon source, such as starch (Sengmee et al. 2017).

H_2 can be obtained from microalgal biomass fermentation by microorganisms that produce it, or as a result of metabolic reactions of microalgal cells (Fig. 17.5). H_2 production by green microalgae is carried out during the photosynthesis process through direct or indirect water biophotolysis. Direct biophotolysis occurs in the presence of sunlight, which is captured by photosystems I and II for oxygen photosynthesis. In this process, H_2 is directly generated from breaking down the water molecule with subsequent O_2 release. In indirect biophotolysis processes, H_2 generation occurs from the carbohydrate (starch) molecule breakdown, which is previously synthesized by a biological system in the presence of water and CO_2 that is absorbed from the atmosphere. Thus, carbohydrate breakdown generates H_2 and CO_2. One of the major challenges of H_2 production by microalgae is an incompatibility between oxygen photosynthesis and anaerobic H_2 production, due to fact that hydrogenase is highly sensitive to O_2 (Benemann 2000; Khetkorn et al. 2017; Kruse and Hankamer 2010; Márquez-Reyes et al. 2015).

The microalgae *C. reinhardtii* has been used as model for studying H_2 production, although studies with other species are underway (Table 17.3). In addition, the most widely used culture medium for H_2 production by microalgae has been the TAP medium, as shown in Table 17.3. H_2 photoproduction can be favored by sulfur and phosphorus deprivation in freshwater algae cultures, and by phosphorus deprivation in seawater algae cultures (Batyrova et al. 2015; Dasgupta et al. 2015; Sengmee et al. 2017).

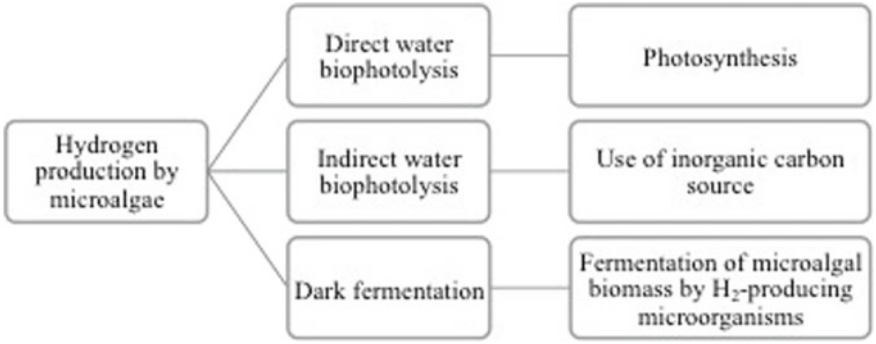

Fig. 17.5 H_2 production processes from microalgae

17.2.3 Biodiesel Production from Microalgal Biomass

The main advantage of using biodiesel as fuel instead of regular diesel lies in CO_2 emissions. With respect to biodiesel, these emissions can be considered as carbon credits, since plants and microalgae use CO_2 as an inorganic carbon source for their metabolic reactions. In addition, biodiesel can be used as fuel for generating the power necessary to produce and process microalgae (Chisti 2008).

Under unfavorable environmental conditions (stress conditions) generated by nutrient deficiency or amount of light, microalgal cells accumulate lipids in the form of triacylglycerides (Widjaja et al. 2009). Biochemical changes, such as low nitrogen supply, may reduce cell proliferation due to the scarcity of proteins that participate in cell wall formation (Aremu et al. 2015; Ördög et al. 2012). On the other hand, phosphate deprivation can negatively affect biomass production without significant losses in lipid concentration, although the concentration of unsaturated fatty acids increases significantly, which is uninteresting with respect to biodiesel production (Praveenkumar et al. 2012). Moreover, supplying organic carbon sources stimulates cell growth and lipid accumulation by the microalgal cell (Li et al. 2011).

The steps for obtaining biodiesel from microalgae culture are shown in Fig. 17.2. Triacylglycerols present in the oil extracted with the aid of solvents are cleaved in consecutive steps of reaction with methanol (methanolysis) in diglycerides and monoglycerides. Other short-chain alcohols, such as ethanol, may also be effective in this process. Acidic, basic and enzymatic catalysts or even supercritical conditions can be used, which results in increased process efficiency. In the final stages, fatty acid methyl esters (FAME) or fatty acid ethyl esters (FAEE) and glycerol (reaction byproduct) are obtained (Gong and Jiang 2011).

Solvents which are often used in the extractive process may not fully solubilize triacylglycerols, thus rendering the process inefficient and resulting in reduced oil extraction rate (Velasquez-Orta et al. 2013). In addition, biomass drying temperature can significantly interfere with oil recovery, since high temperatures promote the

oxidation of fatty acids (WIDJAJA et al. 2009). Therefore, adequate oil extraction techniques from microalgal cells are essential for good biodiesel yields.

17.2.4 Bioethanol Production from Microalgal Biomass

Bioethanol is a liquid fuel that can be produced with sugars extracted from vegetable raw material. They represent an attractive alternative to fossil fuels because they are obtained from renewable sources, thus the process of obtaining ethanol is considered a green technology. In addition, ethanol has higher octane rating, flammability limits and flame speed in comparison with gasoline, thence allowing high compression ratio and lower burning rate. The presence of oxygen in the molecular structure of ethanol improves combustion, thereby reducing particulate, hydrocarbon and carbon monoxide emissions (Balat et al. 2008).

According to Hernández et al. (2015) cell composition that is rich in lipids and proteins with low carbohydrate content, it is uninteresting to conduct research aimed at obtaining bioethanol from microalgae. In fact, obtaining ethanol from microalgal biomass has not been studied as widely as biodiesel production, although ethanol production from vegetable biomass has been increasing considerably over the years (Reyimu and Ozçimen 2017). Chen et al. (2013) classifies microalgae as a promising carbohydrate source, whose cell wall is rich in polysaccharides such as cellulose, hemicellulose, glycoproteins, pectin, agar and alginate, as well as the ability to accumulate starch. In this context, species with higher potential for carbohydrate accumulation should be used and culture supplementation should be evaluated (Dragone et al. 2011) to convert sugar into ethanol. Examples of promising microalgae species for such a purpose are listed in Table 17.4.

The steps for obtaining ethanol from microalgae are illustrated in Fig. 17.2. Structural and reserve polymeric carbohydrates must be broken down, and released monomers should be converted into ethanol by specific microorganisms. Although microalgae cell wall does not present lignin in its composition, the use of pre-treatments to disorganize its structure and expose structural and reserve sugars has been studied in order to increase ethanol yield (Chng et al. 2017). Pre-treatments have widely been used to reduce the recalcitrance of lignocellulosic materials for 2G biofuel production (Alvira et al. 2010). Chemical and physicochemical pre-treatments using acid, alkali, ozone or solvents, followed by enzymatic hydrolysis, have been reported in literature (Table 17.4). According to Keris-Sen and Gurol (2017) and Chng et al. (2017), Organosolv and Ozonolysis presented the highest sugar release rate when compared to other studied pre-treatments, thus obtaining higher ethanol production rate.

Microorganism fermentation for converting fermentable sugars into ethanol can be carried out by means of separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF). In SHF, the microorganism is added to the reaction medium that has been previously supplemented with necessary nutrients for its growth after carbohydrate hydrolysis. In this case, hydrolysis and fermenta-

tion are performed in two steps. The disadvantage of this process is the possibility of microorganism inhibition by the substrate and the long time required to obtain monomers and ethanol. In SSF, enzymes and the microorganism are incubated in the same reaction medium. As sugar monomers are released, the microorganism uses sugars as carbon source for obtaining energy. In this model, ethanol is produced while monomers are released simultaneously, and substrate inhibition is practically zero. Furthermore, total reaction time is reduced when compared to SHF (Chng et al. 2017; Ho et al. 2013). The most widely studied microorganisms that convert microalgae glucose into ethanol are *Saccharomyces cerevisiae* and *Zymomonas mobilis*, according to Table 17.5.

17.3 Pigment Production from Microalgal Biomass

Natural pigments can be produced by plants and microorganisms to be used in food, pharmaceutical and cosmetic industries. They are called natural dyes and exhibit greater sensitivity to light, temperature and oxide-reducing agents when compared to synthetic dyes. These factors contribute to a reduced use of natural pigments, thus microencapsulation techniques can be used in order to improve their stability (Özkan and Bilek 2014).

Among photosynthetic pigments produced by microalgae, chlorophylls (green), carotenoids (red, orange and yellow) and phycobilins (red and blue) are the three major classes (Begum et al. 2016). Their color is a result of a conjugate system of double bonds in the molecule structure. Electron excitation in these bonds is followed by the absorption of specific wavelengths. As a consequence, the light that is not absorbed by the molecule is reflected, thence coloring the cells (Mulders et al. 2014). For this reason, pigments are used as a criterion for microalgae classification (Serive et al. 2017).

Pigment synthesis by microalgae has been described by Mulders et al. (2014) and can be affected both by culture conditions (light, temperature, pH and salinity) (Begum et al. 2016) and by the culture medium supplementation. Examples of culture media used to obtain pigments from microalgae and supplementation are listed in Table 17.6. However, the extractive process may influence their recovery rate (Faraloni and Torzillo 2017; Ferreira and Sant'anna 2017).

17.3.1 Chlorophyll

Green microalgae have been extensively studied as raw material for biofuel production. These algae have high concentrations of chlorophyll, characterizing their coloration. Chlorophylls are essential for photosynthesis and are capable of absorbing light and converting it into chemical energy and its increased concentration in the culture medium is a sign of cellular proliferation (Benavente-Valdés et al. 2017). The

Table 17.5 Culture media, types of photobioreactors and cultures to obtain sugars and ethanol from different microalgae species

Microalgae strain	Culture media	Reactor	Culture condition	Culture type	Agitation	Process to obtain sugars	Fementation strain	Maximun ethanol yield	References
C. reinhardtii UTEX 90	TAP	PBR (2.5 L)	PA	Indoor	Ns	Liquefation and sacchaarification (α-amylase from *B. licheniforms* and amylosglucosidase from *Aspergillus niger*)	*S. cerevisiae* S288C	235 mg of ethanol was obtained from the hydrolyzed starch of 1.0 g algal biomass	Choi et al. (2010)
Chlorococum sp.	Author medium	Bag (100 mL)	PA	Outdoor	Bubbling	Cells subjected to supercritical lipid extraction	*S. cerevisiae*	3.83 g L^{-1} ethanol obtained from 10 g L^{-1} of lipid-extracted microalgae debris	Harun et al. (2010)
Chlorococum humicola	Author medium	Bag (100 mL)	PA	Outdoor	Ns	Enzymatic hydrolysis (cellulase from *Trichoderma reesei*)	*S. cerevisiae*	–	Harun and Danquah (2011a)
					Bubbling	Acid pre-treatment (H_2SO_4 1–10% (v/v))		7.20 g ethanol/L hydrolyzate	Harun and Danquah (2011b)
	Ns	Ns	Ns	Ns	Ns	Alkaline pre-treatment (NaOH0.75% (w/v))		26.1% g ethanol/g algae	Harun et al. (2011)
C. vulgaris FSP-E	Modified Basal medium	Glass vessel (1L)	PA	Indoor	Ns (300 rpm)	Enzymatic hydrolysis (hydrolytic enzymes from *Pseudomonas* sp. CL3)	*Z. mobilis*	79.9% (SHF) and 92.3% (SSF) theoretical yield	Ho et al. 2013
						Acid pretreatment (H_2SO_4 1–5% (v/v))		87.6% (SHF) theoretical yield	
Scenedesmus dimorphus UTEX 1237	Author medium	PBR (2 L)	PA	Indoor	Aeration	Organosolv pretreatmet	*S. cerevisiae* YSC2	>90% (SSF)	Chng et al. (2017)
						Acid pretreatment (H_2SO_4 4% (v/v))		80% of theoretical yield (SSF)	

(continued)

Table 17.5 (continued)

Microalgae strain	Culture media	Reactor	Culture condition	Culture type	Agitation	Process to obtain sugars	Fementation strain	Maximun ethanol yield	References
						Enzymatic hydrolysis		84% of theoretical yield (SSF)	
Scenedesmus obliquus CNW-N	Modified Detmer's Medium	PMMA-made tubular (60 L)	PA	Outdoor	Aeration (0.06 vvm)	Acid pretreatment (H_2SO_4 2% (v/v))	*Z. mobilis* ATCC 29191.	0.205 g ethanol/g biomass	Ho et al. (2017)
N. oculata	Municipal wastewater and seawater	Erlenmeyer flasks (250 mL)	PA	Indoor	Shaking incubator (150 rpm)	Alkaline pretreatment (NaOH 0.75% (w/v))	*S. cerevisiae*	Bioethanol yield of *N. oculata* and *T. suecica* ranged from 0.41% to 7.26%	Reyimu and Ozçimen (2017)
Tetraselmis suecica									
Mixed microalgal culture containing species from the *Chlorococcales* order of the *Chlorophyceae* class	BG11 medium	Glass tanks (20L)	PA	Indoor	Aeration (CO_2)	Ozonolysis (0.25–2 g O_3/g of dry weight biomass)	–	–	Keris-Sen and Gurol (2017)
						Ultrasound (ultrasonic energy intensity of 1.6 kWh/gram of biomass)			
						Alkaline (NaOH 0.75–2% (w/v))			
						Acid (H_2SO_4 concentrated)			

PBR photobiorreator; *PA* photoautotrophic; *PH* photoheterotrophic; *MT* mixotrophic; *Ns* not specified

Table 17.6 Influence of supplementation and culture conditions on pigment production from different microalgae species

Microalgae	Culture media	Reactor	Culture condition	Nutrient	Agitation	Composition/productivity	References
Chlamydomonas moewsii	Bristol modified	Pyrex glass bottles and	PA	Sodium sulphate	Aeration	Maximum production of chlorophyll a and chlorophyll b was observed at concentrations ranging between 0.1 and 3 mM of sodium sulfate. Concentrations above 5 mM exerted an inhibitory effect	Mera et al. (2016)
Trachydiscus minutus	Mineral media	Glass cylinders	PA	Ce^{3+}, Gd^{3+}, La^{3+}, Pr^{3+}, Sc^{3+}, Lu^{3+} and monazite.	Aeration CO_2 2% (v/v)	There was no significant influence of metals on pigment production	Goecke et al. (2017)
Parachlorella. kessleri						It was observed a marked decrease in the production of lutein (Lu^{3+} and PR^{3+}), chlorophyll a (Lu^{3+}, PR^{3+} and Mon), chlorophyll b (PR^{3+} and Mon) and β-carotene (Gd^{3+}, La^{3+}, Pr^{3+}, Sc^{3+}, Lu^{3+} and Mon) Ce^{3+}, Gd^{3+}, La^{3+} and Sc^{3+} have increased carotenoid and chlorophyll production; monazite increased violaxanthin production	
Porphyridium cruentum	F/2	Tubular PBR	PA	–	Bubble column and liquid circulation with the aid of a centrifugal pump	Chlorophyll a and carotenoid contents were positively affected by the drying temperature (170–190 °C) 415.88 μg/g of β-carotene and 1513.12 μg/g of chlorophyll were obtained when biomass was dried at 180 °C; higher recovery than drying at room temperature	Durmaz (2017)
C. vulgaris	Culture media for heterotrophic condition	Flat panel airlift	PA	–	Aeration	Cell growth and pigment production (carotenoids and chlorophyll) were observed in both conditions, although cell proliferation and subsequent pigment production were larger in the photoheterotrophic condition	Benavente-Valdés et al. (2017)
		Flat panel airlift and stirred tank	PH		Aeration and mechanical agitation		
		Flat panel airlift	MT		Aeration		

PBR photobiorreator; *PA* photoautotrophic; *PH* photoheterotrophic; *MT* mixotrophic

chlorophyll molecule consists of an aromatic ring, called chlorine, which contains 4 pyrrole rings surrounded by a magnesium ion. A hydrocarbon tail (phytol) can be found attached to chlorine (Mulders et al. 2014). According to Chen et al. (2010), there are 5 types of chlorophyll: *a*, *b*, *c*, *d* and *f*. Although these groups exhibit similar molecular structures, they have differences in their macrocyclic peripheral groups, thus causing their light absorption spectrum to be different.

Microalgae require favorable conditions for photoautotrophic growth, including light, water, inorganic carbon (CO_2), inorganic nitrogen (ammonia or nitrate) and phosphate. The availability of these nutrients significantly affects chlorophyll production by microalgal cells (Mulders et al. 2014). Reduced concentrations of nitrogen, sulfur, iron, magnesium and phosphorus or high concentrations of copper and zinc may reduce chlorophyll synthesis. In addition, reduced light supply limits the conversion of inorganic carbon into organic molecules, thereby limiting growth and energy uptake by cells (Ferreira and Sant'anna 2017; Mulders et al. 2014).

The process of chlorophyll extraction from microalgal cells can be observed in Fig. 17.3 and resembles lipid extraction due to the use of solvents and the need for cell disruption. The cold extraction process has been widely used, since pigments are sensitive to high temperatures. No reports of chlorophyll extraction were found in literature.

The first step for extracting pigments is cell disruption, where the dried cells are immersed in a polar solvent and the resulting suspension is incubated under ultrasound irradiation. Authors have reported the use of acetone (90–100%) (D'este et al. 2017; Mera et al. 2016; Van Heukelem and Thomas 2001), ethanol (Bertrand et al. 2002; Lv et al. 2017; Serive et al. 2017; Van Heukelem and Thomas 2001) and methanol (Goecke et al. 2017). It is recommended to use an ice bath to maintain low temperatures as a way to prevent the degradation of extractives. The extracted pigments can be quantified by spectrophotometry, using specific wavelength for each pigment (Durmaz 2017; Lv et al. 2017; Mera et al. 2016), fluorescence (Lv et al. 2017) or by high-performance liquid chromatography (HPLC) (D'este et al. 2017; Goecke et al. 2017; Serive et al. 2017; Van Heukelem and Thomas 2001).

17.4 Conclusion

Microalgal biomass represents an attractive alternative to oil for fuel obtaining once it is classified as a renewable feedstock. Different types of biofuels can be obtained from microalgal biomass in separate or co-generation process such as biodiesel, bioethanol and biohydrogen. Those biofuels are classified as green fuels due to the lower contribution to the greenhouse effect when compared to fossil fuels. Besides biofuels, microagal biomass is also a source of pigments, such as chlorophyll, which are value-added bioproducts and shows a wide range of uses in textile, pharmaceutical, cosmetics industries. In this way, microalgae are a promise feedstock to be processed is a biorefinery concept for energy generation and value-added products obtaining.

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