Chapter 30: Microalgal biorefineries

Alice Ferreira[a], Luisa Gouveia[a,*]

[a]*LNEG, National Laboratory of Energy and Geology I.P., Bioenergy Unit, Estrada do Paço do Lumiar 22, 1649-038 Lisbon, Portugal.*

[*]Corresponding author: luisa.gouveia@lneg.pt

Abstract

Microalgae-based bioproducts remain expensive mainly due to microalgae cultivation, harvesting and downstream processing costs. Nonetheless, microalgae are a high potential source of several biofuels, biofertilizers, and bioproducts (e.g. carbohydrates, long-chain fatty acids, pigments and proteins), which can provide important nutritional, cosmetical, pharmaceutical and health benefits. In addition, they are able to perform wastewater bioremediation and carbon dioxide mitigation. This not only contributes to a more sustainable microalgae production, with environmental benefits, but offers cost savings on the whole process. Hence, from these small cellular factories, a large source of compounds and products can be obtained, providing a real microalgal-based biorefinery. This type of approach is crucial for the full application and commercialization of microalgae in a large range of products and industries, with added benefits for bio-economy and society in general.

This chapter addresses the potential transformation of microalgal biomass into a wide range of marketable products, presenting examples of experimental microalgae-based biorefineries grown in an autotrophic mode at a laboratory scale.

Keywords: Microalgae; Carbohydrates; Lipids; Pigments; Biorefinery; Circular Economy

1. Introduction

The search for renewable fuels has gained attention due to the higher energy demand imposed by the ever-increasing world population. In this context, microalgae are now accepted as a significant alternative source for renewable fuels. In addition to the microalgal lipids that could potentially be converted into biodiesel, the microalgal carbohydrates could be used in fermentation processes (to generate bioethanol and/or biohydrogen). Microalgae, besides lipids and carbohydrates also contain many other valuable components, including, polyunsaturated fatty acid, antioxidants and pigments. These compounds could widen the market opportunities of microalgae products and open up further possibilities of coupling production of microalgae for biofuels and high value compounds (Chew et al., 2017).

Microalgae are grown in open ponds or closed systems that involve mixing and concentrating processes. Moreover, the downstream processing, which includes harvesting, drying, compounds extraction and conversion technologies, can be challenging. Altogether, the microalgae production for biofuels is economically unfeasible. One path to drive down the cost of biofuels is to reduce the cost of biomass production (i.e. cultivation/harvesting). However, recent techno-economic analysis work has demonstrated that reducing the costs to a level that would enable biofuel economic viability is extremely challenging (Laurens et al., 2017). Thus, another way for turning economic and energy balances more favorable is to derive multiple products in a single cycle (Biorefinery concept) (Bhalamurugan et al., 2018), and to use effluents as a nutrient source for cultivation

As more promising bioproducts are developed and evaluated, a higher value can be added to the microalgal biomass, thereby lowering the pressure on increasing the productivity to achieve rigorous cost targets. This versatility and huge potential of tiny microalgae could support a microalgae-based biorefinery and microalgae-based bioeconomy opening up vast opportunities in the global algae business (Laurens et al., 2017). The microalgae could play an important response to the worldwide biofuel demand, together with the production of high value-added products and assisting some other environmental issues such as water stream bioremediation and carbon dioxide mitigation (Gouveia, 2015).

2. Biorefinery concept

A biorefinery is a facility (or network of facilities) that integrate biomass conversion processes and equipment to produce transportation biofuels, energy, and high-value products from biomass. The concept of biorefinery is similar to a traditional petroleum refinery, in which biomass is converted into multiple marketable chemicals, fuels and products (Chew et al., 2017).

A biorefinery chain includes the pre-treatment and separation of biomass components and the subsequent conversion to generate a spectrum of different intermediates and products. By producing multiple products, a biorefinery can take advantage of the differences in biomass components and intermediates, maximizing the value of the biomass feedstock and preventing resource loss and environmental impacts (Singh and Gu, 2010; Zhu, 2015).

Biorefineries are found in multiple sectors at industrial scale, which allows the concentration of various products processing (Chew et al., 2017).

3. Microalgae-based biorefinery

Microalgae play a major role in the production of biofuels and bio-based chemicals making them a promising alternative to many natural components and sources. Microalgae high value-added products previously cited, can be enhanced under stressed environmental conditions and be used as feedstock for different products. Extracted microalgal lipids can be employed as potential feedstock for biodiesel production while carbohydrates can be used as a carbon source in fermentation industries to replace conventional carbohydrate sources such as simple sugars or lignocellulosic biomass. Moreover, some microalgae contain long-chain polyunsaturated fatty acids (PUFAs), such as Eicosapentaenoic Acid (EPA), Docosahexaenoic Acid (DHA) and Arachidonic Acid (AA), that can act as health food supplements, as well as proteins and pigments that exhibit properties desired by the food, feed, in addition to pharmaceutical industries to treat certain diseases (Chew et al., 2017).

The advantage of using microalgae is the rapid growth rate and high photosynthetic efficiencies with small amounts of water, nutrients and atmospheric CO_2 in comparison to terrestrial plants. It does not create the competition for land and food crops since they can grow on degraded land and marginal areas (Khoo et al., 2011). Another virtue of microalgae is the ability to grow on industrial wastewaters by using their excess nutrients, while simultaneously promoting a more sustainable wastewater treatment. Furthermore, they can sequester the excess CO_2, not only from the atmosphere, but also from anthropogenic flue gases from pollutant industries, such as cement plants and thermoelectric stations, contributing immensely for the reduction of greenhouse gases (GHG) emissions (Cheah et al., 2015). Hence, microalgae have been considered as a sustainable feedstock for the biorefinery industries of the future and some microalgae-based biorefineries have already been developed, as shown in section 3.5.

Nevertheless, there are still several challenges that need to be tackled during the development of microalgae-based biorefinery technologies. The most challenging problems include high investment and operation cost, difficulty in controlling the culture conditions, contamination bacteria or undesired algae, unstable light supply and weather, among others. Several strategies have been proposed to solve these challenges. The selection of the most adequate microalgal strains in terms of target product, tolerance and adaptation capacity to environmental conditions is a very important requirement for stable and sustainable microalgae cultivation. Also, identifying the most advantageous culture conditions and operation design is critical for improving the productivity of microalgae and derived products. Finally, a high-efficiency and low-cost downstream processing (harvesting, drying, extraction, conversion) should be developed. In addition, appropriate treatment of the wastes produced from microalgae systems as well as recycling of water used during microalgae cultivation processes are also critical issues. Finally, life cycle analysis, energy balance and cost assessment should also be performed to justify the economic feasibility and environmental impacts (Yen et al., 2013).

3.1. Wastewater treatment

The combination of wastewater treatment with microalgal cultivation was first highlighted in the 1950s by Oswald and Gotaas (Oswald and Gotaas, 1957). Since then, algal-bacterial systems have arisen as a promising platform to support a sustainable and low-cost wastewater treatment due to the ability of microalgae to grow in nutrient-rich environments together with the accumulation of nutrients from wastewaters, and the need of reducing microalgae production costs (Ferreira et al., 2018; Posadas et al., 2017).

Microalgal-bacterial processes provide an effective treatment for replacing conventional tertiary treatment, with lower associated costs and environmental impacts. They can play a dual role of bioremediation of wastewaters due to their potential for cost-free oxygenation and simultaneous nutrient removal, while producing valuable biomass with concomitant CO_2 sequestration. Furthermore, this microalgae-based remediation allows nutrient recycling into a valuable biomass that can be further processed for different applications, without secondary pollution (Ferreira et al., 2018; Rawat et al., 2011). This strategy represents a double benefit for both parts, since microalgae provides the cleaning of wastewaters, while offering a source of water and nutrients that is readily available and at lower costs (Cuellar-Bermudez et al., 2017).

Microalgal-based wastewater treatment is achieved through photosynthesis, by which micro-algae supply O_2 to heterotrophic aerobic bacteria to mineralize organic pollutants, using in turn the CO_2 released from bacterial respiration. Therefore, this avoids the use of intensive mechanical aeration, reducing operation costs and minimizing pollutant volatilization (Muñoz and Guieysse, 2006).

A wide range of microalgae such as *Chlorella*, *Scenedesmus*, *Phormidium*, *Botryococcus*, *Chlamydomonas* and *Spirulina* were already used for treating different wastewaters with promising results (Ferreira et al., 2018; Gao et al., 2018; Kong et al., 2010; Martínez et al., 2000; Mata et al., 2013; Posadas et al., 2015, 2014; Wang et al., 2010). The studies show that microalgae provide an effective wastewater treatment, while it avoids the use of fresh water and nutrients.

3.2. Carbon dioxide mitigation

The fixation of CO_2 performed by photosynthetic organisms on earth has contributed significantly to the global carbon cycle. The CO_2 produced from natural or human activities can be consumed by plants and algae, converting it into biomass and other metabolic products through photosynthesis and Calvin cycle. Moreover, the CO_2 fixation is accompanied by production of microalgae biomass, which could be converted to a variety of biofuels, pigments, cosmetic, nutritious food and animal feed, representing additional benefits from the microalgae CO_2 fixation process. Since microalgae-based CO_2 fixation is much faster and more efficient (around 10-50 times higher) than that of terrestrial plants, it has thus been considered to have the potential to serve as a commercially feasible process for mitigation of CO_2 emissions (Ho et al., 2011). Most microalgae can fix the dissolved inorganic carbon and CO_2 in the gaseous effluents to form chemical energy through photosynthesis. Their ability to tolerate high CO_2 contents allows them an efficient capture of CO_2 from streams such as flue gases and flaring gases (CO_2 content of 5-15%) (Hsueh et al., 2007).

3.3. Biofuel generation

Microalgae represent a promising alternative based on inherent advantages such as rapid growth rate, high lipid yields, high CO_2 uptake rate, lower land use, lower water consumption, daily harvesting, etc. However, microalgae production remains economically unsustainable. The possibility of coupling wastewater treatment, using nutrients from waste streams (e.g. WWs and/or CO_2 flue gas emissions), with microalgae cultivation is crucial to provide a positive energy return (Lundquist et al., 2010; Pittman et al., 2011). Furthermore, it can bring additional benefits to the reduction of environmental impact and disposal problems (Mata et al., 2013). Efforts have been made in order to advance the commercial feasibility of microalgae derived biofuels, focusing on the improvement of processing steps, from the production of feedstock to fuel conversion processes (Quinn and Davis, 2015).

The conversion technologies for microalgal biomass can be divided into four categories, namely thermochemical conversion, biochemical conversion, transesterification and photosynthetic microbial fuel cell. The main factor affecting the choice of conversion process are the quantity and type of biomass feedstock, economic considerations, specification of projects and the end form of the desired product (Brennan and Owende, 2010).

3.3.1. Thermochemical conversion

Thermochemical conversion consists on the thermal decomposition of organic materials in biomass to extract fuel products (Brennan and Owende, 2010). This includes the processes of gasification, thermal liquefaction, pyrolysis and direct combustion. These conversion techniques are a promising pathway to separate the different microalgal compounds due to their small footprint, shorter processing times, feedstock flexibility, efficient nutrient recovery and no fugitive gas emissions (Ferreira et al., 2015). Furthermore, the high temperatures eliminate possible pathogens and bioactive compounds, leaving only minor residues (Razzak et al., 2013). Gasification is the chemical process where carbonaceous materials are converted to synthesis gas (syngas) at high temperatures (800-1000 °C). Syngas is a mixture of CO, H_2,

CO_2, N, and traces of CH_4. It can be used to make a wide range of fuels and chemical intermediates or it can be directly burnt to be used as a fuel for gas engines. For thermal liquefaction, the algal biomass will undergo liquefaction, at low-temperature (300-500 °C) and high pressure (5-20 MPa). to decompose the biomass into smaller molecules with higher energy density. On the other hand, pyrolysis depicts the thermal degradation of biomass in an oxygen-free atmosphere under 350-700 °C. This process has potential for large scale production and can generate biofuels with medium-low calorific power (bio-oil, bio-char, biogas) (Brennan and Owende, 2010). The pyrolysis gases usually contain CO, CO_2, light hydrocarbons (C1-C4) and H_2. Regarding the bio-char, this presents a high content of C, some H, and a minimum of O. Bio-char can be used in various ways such as a soil amendment, energy carrier, adsorbents and catalyst support. Finally, bio-oil is a complex mixture of oxygenated compounds, water (15 – 40 wt%) and some fine char particles (Fermoso et al., 2017). In a direct combustion, biomass is burnt in the presence of air, producing carbon dioxide, water and heat. Energy is generated through the combustion of biomass and higher efficiencies can be achieved with the co-combustion techniques in coal fired power plants (Brennan and Owende, 2010).

3.3.2. Biochemical conversion

The biochemical conversion illustrates the biological processing of biomass into biofuels for energy production. Examples of biochemical conversion processes include anaerobic digestion, alcoholic fermentation and photobiological hydrogen production. Anaerobic digestion involves the conversion of organic wastes into biogas, which is mainly composed of CH_4 (55-75%) and CO_2 (25-45%). The biogas produced from algal biomass was found to contain high energy value and the energy recovery is comparable to that of the extraction from cell lipids. Due to the rising cost of energy, the anaerobic digestion of biomass is becoming attractive as an alternative for fuel production (Brennan and Owende, 2010; Suganya et al., 2016). As for alcoholic fermentation, biomass materials that contain sugars, starch or cellulose are converted into ethanol through the action of yeasts (Brennan and Owende, 2010). Biological hydrogen (bioH2) can be produced mainly by two routes: photobiologically - biophotolysis of water using green algae and cyanobacteria and photo-decomposition of organic compounds by photosynthetic bacteria (Das and Veziroglu, 2008) - and by bacterial fermentative processes such as dark fermentation. The photobiological hydrogen production occurs due to the split of the water into hydrogen ions and oxygen, through the algae. Firstly, the algae are grown photosynthetically in normal conditions, and subsequently cultured by inducing anaerobic conditions to stimulate hydrogen production. Secondly, the simultaneous production of photosynthetic hydrogen and oxygen gas will take place and these gases will be spatially separated (Chew et al., 2017). Dark fermentation is an indirect technology in which several genera of bacteria (namely *Clostridium* and *Enterobacter*) can use the carbohydrates, proteins, and lipids as substrates to produce H_2, CO_2 and organic acids, through the acidogenic pathway.

3.3.3. Transesterification

Transesterification is the reaction of triglycerides with alcohol (usually methanol) in the presence of a catalyst to produce fatty acid chains (biodiesel) and glycerol. Biodiesel is a mixture of monoalkyl esters of long chain fatty acids (FAME) derived from a renewable lipid feedstock such as algal oil. Microalgal biodiesel is renewable, biodegradable, non-toxic and produces less emissions when compared to petroleum diesel (Brennan and Owende, 2010).

3.3.4. Photosynthetic microbial fuel cell

Microbial fuel cells are bio-electrochemical devices that have the capacity to generate electricity from the biodegradation of organic matter under anaerobic conditions. The integration of microalgal photosynthesis with microbial fuel cells has shown potential in the production of an oxygen rich environment and the removal of CO_2 (Uggetti and Puigagut, 2016). The photosynthetic microbial fuel cell consists of an anode and a cathode separated by a proton exchange membrane. The bacteria in the anode oxidize the organic compounds, producing electrons, which are transferred to the cathode electrode through an external circuit producing electricity. The benefit of this system is that bacteria in the anode can also treat biodegradable wastes. In addition, microalgae in the cathode can fixate CO_2, nitrogen and phosphorus while simultaneously produce a biomass rich in value-compounds which could be used in food, feed, nutraceuticals and supplements. The whole system allows the effluent treatments, production of microalgae valuable biomass and producing bioelectricity, very interesting especially in remote areas (Gouveia et al., 2014).

3.4. Valuable Products Obtained from Microalgae

The recent shift to using microalgae for the production of value-added compounds with high commercial interest, have been increase the demand of research of high content of antioxidants and pigments (carotenoids such as fucoxanthin, lutein, beta-carotene, cantaxanthin and/or astaxanthin and phycobilliproteins) and the presence of long-chain PUFAs (e.g., EPA, DHA) and proteins (essential amino acids methionine, threonine and tryptophan), makes microalgae an excellent source of nutritional compounds (Gouveia, 2015).

Moreover, microalgae have also been screened for new pharmaceutical compounds with biological activity, such as antibiotics, antiviral, anticancer, enzyme inhibitory agents and other therapeutic applications. They have been reported to potentially prevent or reduce the impact of several lifestyle-related diseases (Ebrahimi-Mameghani et al., 2014; Shibata et al., 2007, 2003; Shibata and Sansawa, 2006) with antimicrobial (antibacterial, antifungal, antiprotozoal) and antiviral (including anti-HIV) functions and they also have cytotoxic, antibiotic, and anti-tumour properties as well as having bio-modulatory effects such as immunosuppressive and anti-inflammatory roles (Burja et al., 2007; Singh et al., 2005). Furthermore, algae are believed to have a positive effect on the reduction of cardio-circulatory and coronary diseases, atherosclerosis, gastric ulcers, wounds, constipation, anemia, hypertension, obesity and diabetes (Go et al., 2016; Nuño et al., 2013; Yamaguchi, 1996; Yook et al., 2015).

3.4.1. Lipids

Lipids usually account for approximately 30-50% of their total weight (Chew et al., 2017). Some microalgae can accumulate a high percentage of lipids depending on the environmental conditions they are grown. Stress conditions, such as nitrogen starvation, high temperature, pH shift, high concentration of salts, are required to enhance lipid productivity (Kwak et al., 2016). The higher lipid productivity when compared to other lipid-based energy crops, makes microalgae attractive as a raw material for biodiesel and health food supplements and cosmetic applications (Yeh and Chang, 2012).

3.4.2. Proteins

Proteins account for the major constituents of microalgae, comprising of 50-70% of total composition. They are, therefore, one of the most important products of microalgae biorefineries and can be used for human and animal nutrition (Chew et al., 2017).

3.4.3. Carbohydrates

Microalgae can have a high carbohydrate content which can be easily stored due to its relatively high photo conversion efficiency. Algal carbohydrates are mainly composed of glucose, starch, cellulose and various kinds of polysaccharides. Among these, glucose and starch can be used for bioethanol and biohydrogen production (Batista et al., 2014; Ferreira et al., 2012; John et al., 2011; Karemore and Sen, 2016; Miranda et al., 2012), while polysaccharides have biological functions as storage, protection and structural molecules. Microalgal polysaccharides have the capacity of modulating the immune system and inflammatory reactions, being a promising source of biologically active molecules, such as cosmetic additives, food ingredients and natural therapeutic agents (Chew et al., 2017).

3.4.4. Pigments

Microalgal pigments can be divided in three basic classes: carotenoids (carotenes and xanthophylls), chlorophylls and phycobiliproteins. Chlorophylls and carotenoids are generally fat-soluble molecules whereas, phycobiliproteins are water soluble. These pigments have been used as precursors of vitamins in both food and animal feed (Marques et al., 2011b), additives and coloring agents in food applications, biomaterials and in cosmetic and pharmaceutical industries (Chew et al., 2017).

3.4.4.1. Carotenoids

Carotenoids are fat-soluble pigments that are accessory pigments in plants. The most common algal carotenoids are lutein, astaxanthin, β-carotene, zeaxanthin and lycopene. The microalgae carotenoids have been associated and claimed to reduce the risk of: (1) certain cancers (Gerster, 1993; Lupulescu, 1994; Tanaka et al., 2012; Willett, 1994), (2) cardiovascular diseases (Giordano et al., 2012; Kohlmeier and Hastings, 1995), (3) macular degeneration and cataract formation (Snodderly, 1995; Weikel and Chiu, 2012) and possibly may have an effect on the immune system and may influence chronic diseases (Meydani et al., 1995; Park et al., 2010). The global carotenoids market was valued at $1,577 million in 2017 and is projected to reach $2,098 million by 2025, registering a CAGR of 3.6% from 2018 to 2025 (Allied Market Research, 2019).

Most of the lutein produced commercially is extracted from the petals of the marigold flower. However, microalgae are gaining importance due to higher lutein productivities. Furthermore, microalgae require lower land area and labor when compared to marigold cultivars (Fernández-Sevilla et al., 2010). The amount of lutein produced by microalgae can vary depending on the environmental conditions, namely temperature, pH, light intensity, salinity and nitrogen amount (Guedes et al., 2011). The most common microalgae for producing lutein include *Muriellopsis* sp., *Scenedesmus almeriensis*, *Chlorella protothecoides*, *Chlorella zofingiensis*, *Chlorococcum citriforme*, and *Neospongiococcus gelatinosum* (Fernández-Sevilla et al., 2010). Regarding market price, the cost of lutein from microalga *Scenedesmus almeriensis* was approximately 2.5 US$/g lutein (Molina et al., 2005; Sánchez et al., 2008).

Astaxanthin is carotenoid from the xanthophyll family that acts as a potent antioxidant, having strong anti-aging, sun-proofing, anti-inflammatory and immune systems boosting effects. and skin protector from ultraviolet radiations. Certain microalgae like *Haematococcus pluvialis* (Panis and Carreon, 2016) and *Chlorella zofingiensis* (Guedes et al., 2011) have already been successfully used for producing commercial astaxanthin. For example, for astaxanthin obtained from *Haematococcus pluvialis*, the market value was approximately 1.8 US$/g astaxanthin (Cuellar-Bermudez et al., 2015; Panis and Carreon, 2016; Shah et al., 2016).

β-carotene has been used as a coloring agent, an antioxidant and a vitamin-A supplement. Furthermore, it also possesses antiaging and anticancer properties (Pisal and Lele, 2005). The most commonly used microalgae for the production of β-carotene are *Dunaliella salina*, *Scenedesmus almeriensis*, and *Dunaliella bardawil* (Guedes et al., 2011; Pisal and Lele, 2005). For instance, β-carotene from *Dunaliella* sp. has an approximate market value of 0.3-0.7 US$/g β-carotene (Markou and Nerantzis, 2013).

Zeaxanthin is generally a yellow colored carotenoid mainly used in pharmaceutical, cosmetics and food industry applications (Sajilata et al., 2008). *Scenedesmus almeriensis* and *Nannochloropsis oculate* are the most commonly used microalgae for zeaxanthin production

(Granado-Lorencio et al., 2009; Guillerme et al., 2017). The market value for zeaxanthin produced by *Scenedesmus almeriensis* is around 10 US$/ g zeaxanthin (Granado-Lorencio et al., 2009; The Insight Refinery, 2016).

Lycopene is considered as one of the most influential antioxidants and an effective sunscreen agent. It is also known to possess anticarcinogenic and antiatherogenic properties, reducing the risk of chronic diseases like cancer and cardiovascular diseases (Agarwal and Rao, 2000; Mourelle et al., 2017). An *in vivo* study showed that algal lycopene obtained from *Chlorella marina* exhibited a high antioxidant and anti-inflammatory effect in high cholesterol fed rats (Renju et al., 2014).

3.4.4.2. Chlorophylls

Chlorophylls are lipid-soluble pigments with low polarity (Chew et al., 2017). One or more types of chlorophyll are present in microalgae, but the main types are chlorophylls a, b and c. Due to the structural differences, chlorophyll *a* has blue/green pigment with maximum absorbance from 660 to 665 nm and chlorophyll *b* has green/yellow pigment with maximum absorbance from 642 to 652 nm (Begum et al., 2016).

Chlorophyll is an essential compound not only used as an additive in pharmaceutical but also used in cosmetic products. Chlorophyll *a* has been extensively used as a coloring agent because of its stability. Green microalgae have the highest chlorophyll content among all algae, and it is already commercialized from *Chlorella* species (Chew et al., 2017). On the other hand, *Spirulina platensis* has only chlorophyll *a*, being used as a natural color in food, cosmetic and pharmaceutical products (Begum et al., 2016). Moreover, chlorophyll derivatives can exhibit health promoting activities, such as wound healing and anti-inflammatory properties (Ferruzzi and Blakeslee, 2007). Additionally, Balder et al. (2006) suggested that the consumption of chlorophyll was associated to a decrease in the risk of colorectal cancer.

3.4.4.3. Phycobiliproteins

Phycobiliproteins are the major photosynthetic accessory pigments in cyanobacteria and red algae. These include phycocyanin, allophycocyanin, phycoerythrin and phycoerythrococyanin (Sekar and Chandramohan, 2008). Phycobiliproteins are used commercially as natural dyes and fluorescent agents, but also in pharmaceutical (antioxidant, anti-allergic, anti-inflammatory, neuroprotective and hepaprotective agents) and cosmetic industries (perfumes and eye-make up powders). The major sources of phycobiliproteins are *Arthrospira* (*Spirulina*) sp., *Arthrospira platensis*, and *Amphanizomenon floa-aquae* (de Jesus Raposo et al., 2013; Odjadjare et al., 2017).

3.4.5. Polyunsaturated fatty acids (PUFAs)

PUFAs such as DHA and EPA are widely recognized as essential compounds in human health. The increasing demands for PUFAs has motivated the replacing of fish oil for microalgae as source of DHA and EPA, playing an increasing role in the food industry due to the depletion of marine resources (Wang et al., 2015). DHA-rich algal oil is usually obtained from microalgae such as *Schizochytrium*, *Ulkenia*, *Isochrysis galbana*, *Chlorella pyrenoidosa*, *Chlorella ellipsoidea* and *Crypthecodinium* (Matos et al., 2017; Winwood, 2013).

3.5. Microalgae-based bioplastics

Plastics and their by-products are littering our cities, oceans, and waterstreams, and contributing to health problems in humans and animals. These polymers take many years to decompose because they are hydrophobic and do not undergo the action of microorganisms. These issues have been greatly aggravated due to the economic growth from developed and developing countries and the increase in population. Hence, the need to reduce the amount of discarded plastics and the creation of biodegradable ones, combining practicality and economy issues, are absolutely mandatory. The most common polymers used on the formulation of edible films are proteins (gelatin, casein, wheat gluten and zein), polysaccharides (starch, chitosan) and lipids (waxes), which are used alone or combined. These biopolymers are highly biodegradable and decompose easily into inorganic CO_2 and water (Santacruz et al., 2015). The use of vegetable raw materials could be a very favorable alternative, being the microalgae one of the demanded feedstocks.

3.6. Microalgae-based biofertilizers

The continuous use of arable land for cultivation has led to loss of essential nutrients, such as nitrogen and phosphorus, in the soil. Thus, fertilizers play a vital role in improving agriculture to achieve maximum yields. The intensive use of chemical fertilizers and pesticides in agriculture practices has led to a overdependency on synthetic agrochemicals, which are not only finite resources and toxic, but their price is rising (Chakhalyan et al., 2008).

Biofertilizers are cost-effective, eco-friendly and renewable resources, that play a major role on the controlled mineralization and fertilization processes (Kawalekar, 2013). Biological fertilizers contain living or latent microorganisms or natural compounds derived from organisms such as algae, bacteria and fungi, that can help in improving soil fertility and stimulating plant growth (Abdel-Raouf et al., 2012). Hence, the use of biofertilizers-based microalgae would provide a possible solution. In addition, preliminary results using microalgae biomass grown in effluents strongly suggest an important biostimulant capacity, that could have a significant impact on plant growth and seed germination indexes when applied to soil with minimal pre-treatment (Gouveia et al., submitted). A special attention should be given algal biomass and/or microalgal extracts (Michalak et al., 2017, 2016). Microalgae biomass are also known to act as a pesticide, protecting plants from diseases, insects, and abiotic stress (e.g., high salinity, drought, and frost), being thus an alternative to chemical pesticides (Khan et al., 2019).

Most cyanobacteria are capable of fixing atmospheric nitrogen and can be effectively used as biofertilizers (Bhalamurugan et al., 2018). Some studies have already been developed using microalgae as biofertilizer, achieving promising results in seed germination, plant growth and production of flowers, increase in pigments and soil fertility (Agwa et al., 2017; Dineshkumar et al., 2018; Faheed and Fattah, 2008; Garcia-Gonzalez and Sommerfeld, 2016; Renuka et al., 2016; Song et al., 2005). These studies suggest that microalgae are an efficient, economical and safe biofertilizer to substitute chemical fertilizers in enhancing plant growth and having no detrimental effect on the plant.

4. Examples of microalgae-based biorefinery

4.1. *Nannochloropsis* sp. biorefinery

Nobre *et al.* (2013) developed a biorefinery surrounding *Nannocloropsis* sp. microalga with the extraction of value-added compounds such as carotenoids and fatty acids (namely EPA) for food and feed purposes, as well as lipids for biodiesel production. The fractionated recovery of these compounds was done by Supercritical Fluid Extraction (SFE) using CO_2 and ethanol as an entrainer. After the extraction process, the biomass leftovers were used as substrate for *Enterobacter aerogenes*, in a dark fermentation process, to produce bioH_2 (Fig.30.1). The maximum bioH_2 yield was 60.6 mL H_2/g alga (Nobre et al., 2013).

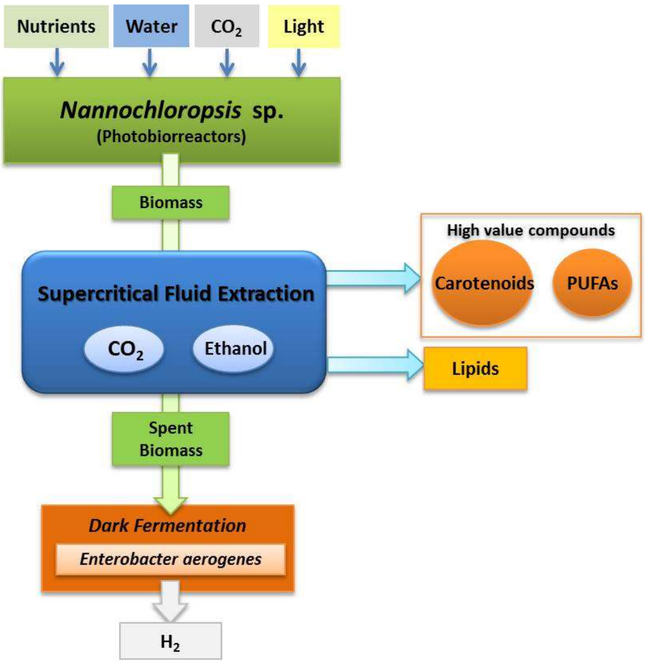

Fig 30.1. *Nannochloropsis* sp. Biorefinery (adapted from Nobre et al., 2013).

Ferreira et al. (2013) did a Life Cycle Inventory (LCI) of the whole process (microalgae cultivation, dewatering, milling, extraction and H_2 production), evaluating the energy consumption, CO2 emissions and economic factors. The authors showed and analyzed five possible pathways and two biorefineries (Fig. 30.2).

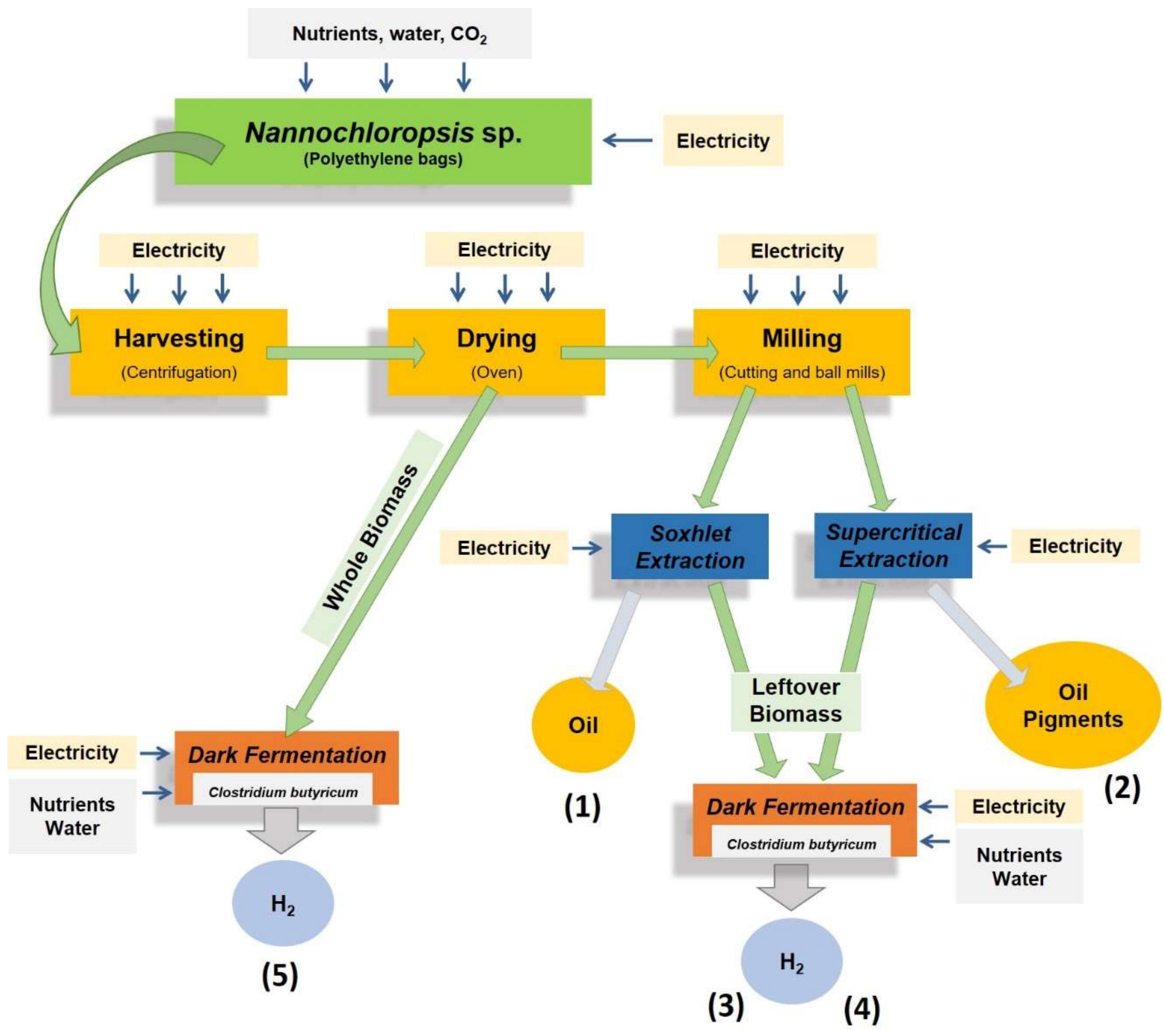

Fig 30.2. *Nannochloropsis* sp. possible biorefineries: Pathway 1 and 3 represent Biorefinery 1, pathways 2 and 4 are Biorefinery 2, and pathway 5 is the direct bioH2 production (adapted from Ferreira et al., 2013).

The analysis of pathways 1, 2 and 5 considers a system boundary that includes the *Nannochloropsis* sp. microalgal culture and the final product output (fatty acids, pigments, or bioH2, respectively). For the remaining pathways, 3 and 4, the bioH_2 production from the leftover biomass from SE and SFE, respectively, was evaluated. The authors concluded that the oil production pathway by SE (3) achieved the lowest energy consumption (176-244 MJ/MJ_{prod}) and CO_2 emissions (13-15 kg CO_2/MJ_{prod}). However, the biorefinery considering the production of oil, pigments and H_2 via SFE was the most economically viable.

From the net energy balance and the CO_2 emission analysis, Biorefinery 1 (biodiesel SE + bioH_2) presented the better results. Biorefinery 2 (biodiesel SFE + bioH_2) showed results in the same range of those in Biorefinery 1. However, the use of SFE produced high-value pigments in addition to the fact that it is a clean technology which does not use toxic organic solvents.

Therefore, Biorefinery 2 was the best in terms of energy, CO_2 emissions and it being the most economically advantageous solution (Ferreira et al., 2013).

4.2. Anabaena sp. biorefinery

The experimental biohydrogen production by photoautrotophic cyanobacterium *Anabaena* sp. was studied by Marques et al. (2011). Hydrogen production from the *Anabaena* biomass leftovers was also achieved by fermentation through the *Enterobacter aerogenes* bacteria and was reported by Ferreira et al. (2012) (Fig. 30.3).

Different culture conditions and gas atmospheres were tested in order to maximize the autotrophic bioH_2 yield versus the energy consumption and CO_2 emissions. The authors stated that the best conditions included an Ar+CO_2+20% N_2 gas atmosphere and medium light intensity (384 W) (Ferreira et al., 2012). The yielded H_2 could be increased using the biomass leftovers through a fermentative process; however this would mean higher energy consumption as well as an increase in CO_2 emissions.

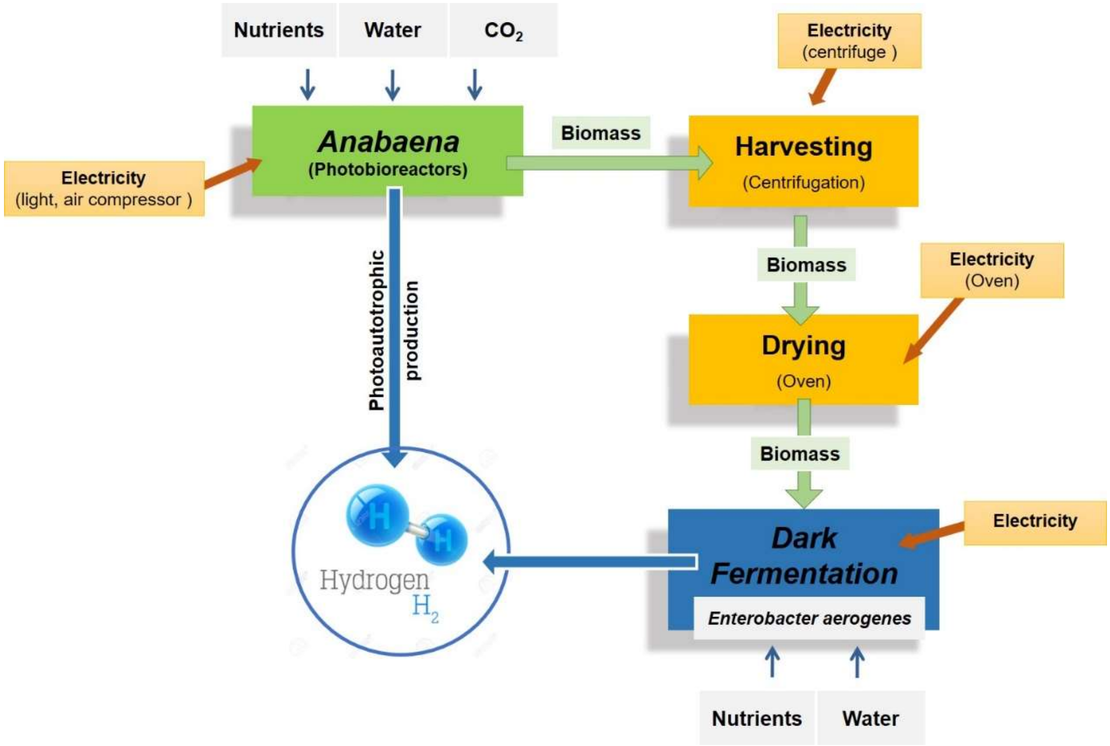

Fig. 30.3. *Anabaena* sp. biorefinery: production of biohydrogen through two pathways (autotrophically and by dark fermentation with *Enterobacter aerogenes*) (adapted from Ferreira et al., 2012).

4.3. Chlorella vulgaris biorefineries

Chlorella vulgaris is one of the most intensively researched microalgae. Therefore, there are a lot a work done concerning biorefinery from this microalga.

Collet et al. (2011) worked on a biorefinery using *Chlorella vulgaris* with lipid extraction followed by methane production from the remaining biomass. The authors developed a Life Cycle Assessment (LCA) and demonstrated that the microalgal methane is the worst case, when compared to microalgal biodiesel and diesel, in terms of abiotic depletion, ionizing radiation, human toxicity, and possible global warming. These negative results are mainly due to a strong demand for electricity. For the land use category, algal biodiesel also had a lesser impact compared to algal methane. However, algal methane is a much better option regarding acidification and eutrophication.

Another work by Ehimen et al. (2011) consider the simultaneous production of biodiesel and methane in a biorefinery concept. The authors obtained biodiesel from a direct transesterification process on the *Chlorella* biomass, and methane through anaerobic digestion of the biomass residues. The maximum methane concentration obtained was 69% (v/v) with a specific yield of 0.308 m^3 CH_4/kg VS, at 40°C and a C/N mass ratio of 8.53. The biodiesel yield was not reported (Ehimen et al., 2011).

Gouveia et al. (2014) studied the simultaneous production of bioelectricity and added-value pigments with wastewater treatment. Fig. 30.4 represents the Photosynthetic Algal Microbial Fuel Cell (PAMFC), where *Chlorella vulgaris* is present in the cathode compartment and a bacterial consortium in the anode compartment. The authors proved that the light intensity increases the PAMFC power and increases the carotenogenesis process in the cathode compartment. The maximum power produced was 62.7 mW/m^2 with a light intensity of 96 μE/(m^2.s).

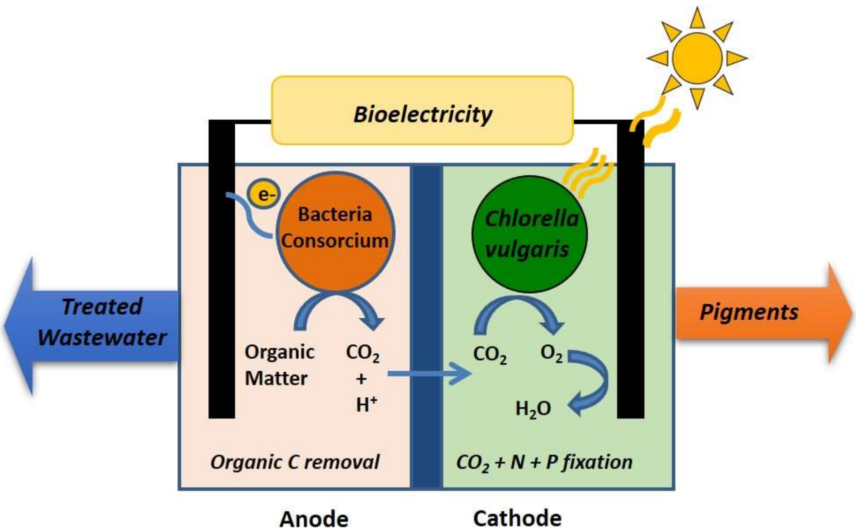

Fig. 30.4. *Chlorella vulgaris* biorefinery: Photosynthetic Algal Microbial Fuel (Gouveia et al., 2014).

Another example of a *C. vulgaris* biorefinery is a bioethanol-biodiesel-microbial fuel cell as reported by Powell and Hill (2009). This fuel cell consisted in an integration of *C. vulgaris* (in the cathode) that captures the CO_2 emitted by yeast fermenters (in the anode). The study demonstrated the possibility of generating electrical power and oil for biodiesel, in a bioethanol production facility. After oil extraction, the remaining biomass could be used for animal feed supplementation (Powell and Hill, 2009).

4.4. *Chlorella protothecoides* biorefinery

The biorefinery developed by Campenni' et al. (2013) consisted in the extraction of lipids and carotenoids from *Chlorella protothecoides* grown autotrophically and with nitrogen deprivation and the addition of a 20 g/L NaCl solution. The leftover biomass could be used for hydrogen or bioethanol production, as the residue still contains sugar (Fig. 30.5).

The total carotenoid content was 0.8% (w/w), which includes canthaxanthin (23.3%), echinenone (14.7%), free astaxanthin (7.1%) and lutein/zeaxanthin (4.1%), that can be used for food applications. Moreover, the total lipid content reached 43.4% (w/w), with a favorable fatty acid composition that complies with the biodiesel EN 14214 quality specifications (European Standard EN 14214, 2004) and can be used for the biodiesel industry.

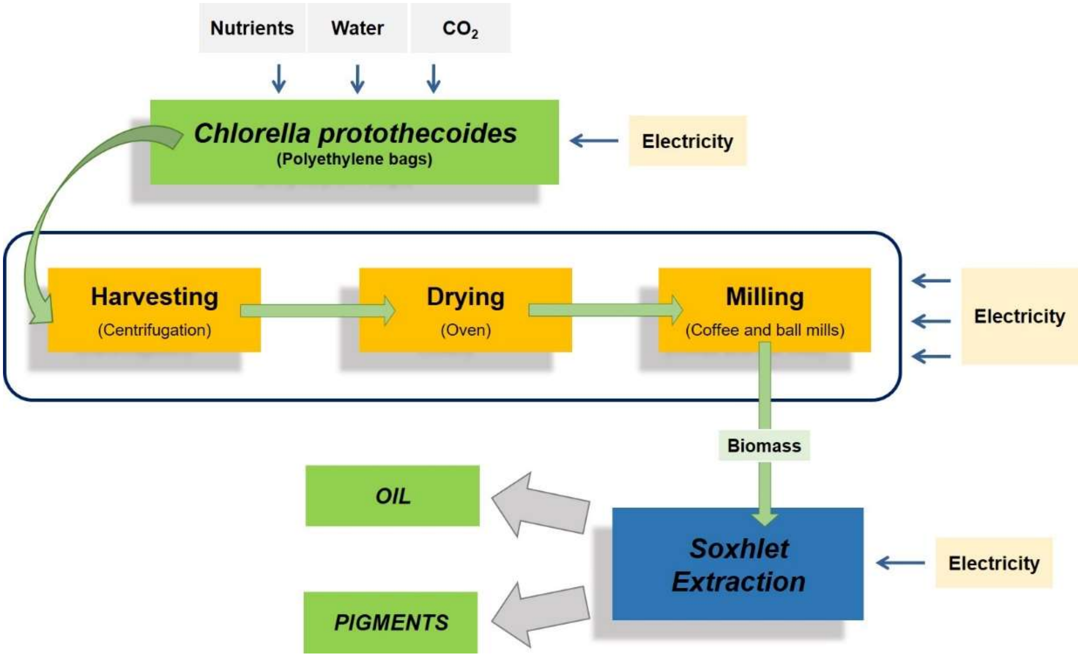

Fig. 30.5. *Chlorella protothecoides* biorefinery (adapted from Campenni' et al., 2013).

4.5. Chlamydomonas reinhardtii biorefinery

Mussgnug et al. (2010) studied the production of biohydrogen from *Chlamydomonas reinhardtii* followed by biogas (methane) production by anaerobic fermentation of the leftover biomass.

The authors verified that using the biomass after hydrogen production instead of the fresh biomass, increased the biogas production around 120%. Thus, the authors concluded that these results were due to the storage compounds with high fermentative potential, such as starch and lipids, which are the key to microalgae-based integrated processes for value-added applications (Mussgnug et al., 2010).

4.6. Dunaliella salina biorefinery

Sialve et al. (2009) showed the production of methane from *Dunaliella salina* after oil extraction for making biodiesel. For shorter hydraulic retention time (HRT, 18 days), the authors achieved a much higher yield (up to 50%), comparing to the values reported by Collet et al. (2011) using *Chlorella vulgaris*.

4.7. *Dunaliella tertiolecta* biorefinery

Lee et al. (2013) investigated the integration of chemoenzymatic saccharification and bioethanol fermentation after lipid extraction of *Dunaliella tertiolecta* biomass for biodiesel production. The bioethanol production achieved yield of 0.14 g ethanol/g residual biomass and 0.44 g ethanol/g glucose. According to the authors, this strategy could improve the economic feasibility of a microalgae-based integrated process.

4.8. *Arthrospira* (*Spirulina*) biorefinery

Olguín (2012) studied a biorefinery with the double purpose of producing oleaginous microalgae grown in wastewater and *Arthrospira* grown in seawater supplemented with anaerobic effluents from animal waste for the production of biofuels (biogas, biodiesel, biohydrogen) and high-value products (PUFAs, phycocyanin, and fish feed). This study highlighted that the biorefinery strategy offers new opportunities for cost-effective and competitive production of biofuels along with nonfuel applications.

4.9. *Spirogyra* sp. biorefinery

Pacheco et al. (2015) did a biorefinery from the sugar-rich microalga *Spirogyra* sp. for the production of bioH_2 and pigments (Fig. 30.6). The authors developed an economic and Life Cycle Analysis of the whole process and concluded that the sugar content of the microalgae must be increased in order to achieve higher bioH2 yields.

The potential energy production and food-grade protein and pigments revenue per cubic meter of microalga culture per year was estimated on 7.4 MJ, US $412 and US $15, respectively, thereby contributing to the cost efficiency and sustainability of the whole bioconversion process (Pinto et al., 2018). Moreover, the use of alternative methods for harvesting and dewatering as well as pigment extraction is crucial to increase the economic viability of the process. The electrocoagulation and solar drying were used in this study and were able to reduce the energy requirements by 90% (Pacheco et al., 2015).

Overall, the major energy consumers and CO_2 emitters of the process was the centrifugation of the microalgal biomass and heating for the fermentation. Pigment production thus becomes necessary to improve the economic benefits of the biorefinery. Nonetheless, it is mandatory to reduce the extraction energy requirements.

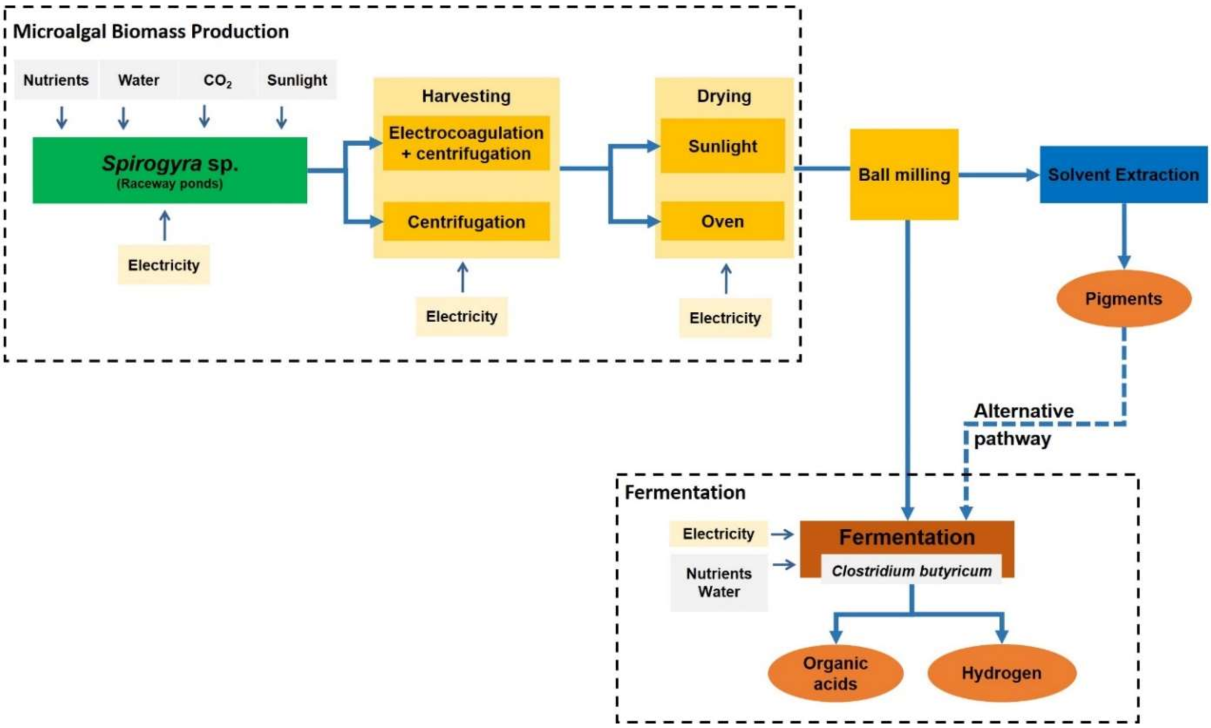

Fig. 30.6. *Spirogyra* sp. biorefinery (adapted from Pacheco et al., 2015).

4.10. Scenedesmus obliquus biorefinery

Ferreira et al. (2019) used microalga *S. obliquus* to successfully treat wastewater from the brewery industry. Furthermore, it used the obtained biomass for different applications, including biofuel production (bioH_2 and pyrolytic bio-oil), subcritical water extraction (SWE) of bioactive compounds (e.g. phenols, flavonoids) and biofertilizers/biostimulants (Fig. 30.7).

The authors achieved high removal efficiencies, obtaining clean waters that respect the imposed limits by Portuguese law (Decree-Law No 236/98, 1998).

SWE of the microalgal biomass allowed recovery yields of 1.016 mg GAE (gallic acid equivalent)/mL for phenols and 0.167 mg CE (catechin equivalent)/mL for flavonoids at 200 ºC. Furthermore, the high temperatures had a sterilizing effect on extracts, which could be beneficial for future food and feed applications.

The wastewater grown *S. obliquus* biomass was also upgraded into biofuel production, achieving a yield of 67.1 mL H_2/g VS for bioH_2 production, and 64% for bio-oil and 30% for bio-char produced from pyrolysis process (Ferreira et al., 2017; Ferreira et al., 2019).

Lastly, the potential of *S. obliquus* biomass was evaluated in barley and wheat seeds and the authors verified that this microalga has an enhancing capacity for plant germination and growth. Moreover, this capacity was further increased when the microalga was grown in brewery wastewater, which is a double benefit for the viability a biorefinery approach.

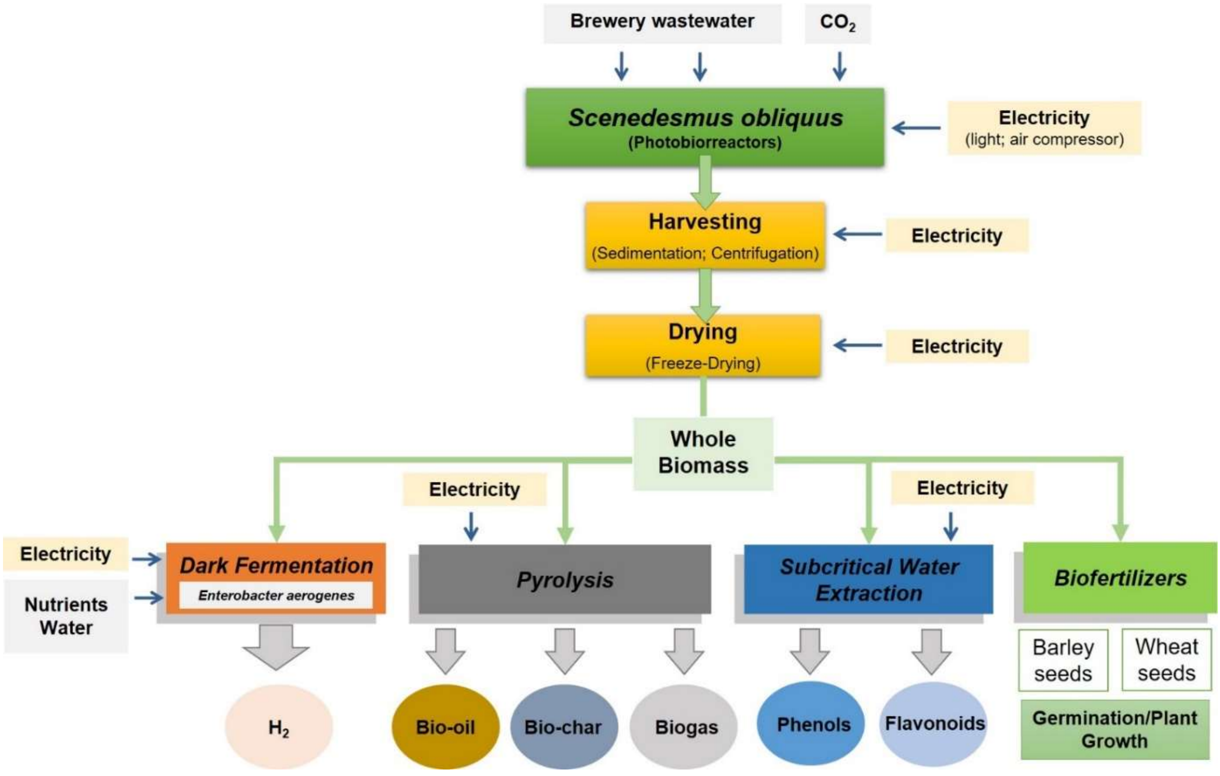

Fig. 30.7. *Scenedesmus obliquus* biorefinery (adapted from Ferreira et al., 2019).

4.11. Tetraselmis sp. biorefinery

Pereira (2019) used *Tetraselmis* sp. CTP4, which is a very robust and tolerant microalga strain to produce a biorefinery which included biofuel and added-value streams.

To minimize harvesting and drying costs, the microalgal biomass was first extracted with ethanol and the crude ethanolic extract was fractionated using a liquid-liquid triphasic system (LTPS). The authors noted the presence of added-value molecules with antioxidant and metal chelating properties, namely phospholipids and carotenoids. The non-polar, water soluble phases, and the leftover biomass from the ethanolic extract was then upgraded into different biofuel applications, namely biodiesel, bioethanol and biogas, respectively. The author obtained a biodiesel mainly compose of palmitic and oleic acid esters, with low amounts of polyunsaturated fatty acid esters. They achieved a bioethanol yield of 0.46 g ethanol/ g fermentable sugar through yeast fermentation after enzymatic hydrolysis. The anaerobic digestion of the residual biomass with or without glycerol supplementation resulted in biomethane yields of 64 and 83%, respectively (Pereira, 2019).

Furthermore, the biochemical composition of the spent biomass also showed to be adequate for food and feed applications (Pereira et al., 2019). Pereira et al. (2020) incorporated the defatted biomass of *Tetraselmis* sp. CTP4 into the feed of juvenile gilthead seabream (*Sparus aurata*), obtaining a similar growth to feed enriched with soybean meal. This means that the defatted microalgal biomass could potentially replace the use of soybean meal in aquaculture feeds, contributing to fulfill the protein demands for EU animal feed market.

Overall, this innovative biorefinery based on *Tetraselmis* sp. CTP4 allowed an effective separation of valuable compounds present in wet microalgal biomass, with the potential for an effective scale-up extraction process.

5. Conclusions

The microalgal business is still very new, but it is currently accepted as a doorway to a multibillion industry, since microalgae are an ecologically safe feedstock for biofuels and products with high commercial value. Furthermore, microalgae have the ability to convert any type of wastewater into a low environmental impact effluent which in turn could serve as a biofertilizer for plants by improving the fertility of the soil and/or to made bioplastic for a cleaner environment. Thus, the sector needs to be further developed to respond to constant demand for eco-friendly innovations to meet the needs of humans regarding food, water and energy.

The implementation of a biorefinery platform for microalgae production is therefore crucial to make exploitation of microalgae cheaper and competitive and support a microalgae-based bioeconomy. A microalgae-based biorefinery should integrate several processes, taking advantage of the various products synthesized by the microalgae for different industries, such as food, feed, energy, agricultural, pharmaceutical, cosmetic, and chemical. Furthermore, it should be done with minimal environmental impact by recycling the nutrients and water (wastewater bioremediation), and by mitigating the CO_2 from the flue gases.

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