Chapter 5
Microalgae Biorefineries for Energy and Coproduct Production

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Abstract The 2015 Conference of the Parties (COP21) marked a turning point for global actions to mitigate atmospheric greenhouse gases, reduce the carbon dioxide emissions from fossil fuel combustion, and stabilize the global climate. On the other hand, the increase in energy demand asks for renewable sources and robust systems to supply energy and obtain product diversity like that obtained from a petroleum refinery. A biorefinery is the sustainable processing of biomass into a spectrum of profitable products and energy. Microalgal biomass is considered one of the most promising biorefinery feedstock providing alternatives for different areas, such as food, feed, cosmetics and health industries, fertilizers, plastics, and biofuels including biodiesel, methane, hydrogen, ethanol. Furthermore, microalgae can also be used for the treatment of wastewater and CO_2 capture. However, microalgal biofuels are not currently cost competitive at large scale and to develop a sustainable and economically feasible process, most of the biomass components should be valorized. High-value coproducts from microalgae include pigments, proteins, lipids, carbohydrates, vitamins, and antioxidants, and they can improve the process economics in the biorefinery concept. Therefore, mild and energy-efficient downstream processing techniques need to be chosen to maintain product properties and value. In this chapter, the existing products and microalgae biorefinery strategies will be presented, followed by new developments, sustainability assessments, and techno-economic evaluations. Finally, perspectives and challenges of microalgal biorefineries will be explored.

Keywords Biorefinery · Downstream processing · Biofuels · Microalgae products
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1 Introduction

The World Meteorological Organization confirmed that 2016 was the hottest year on Earth; the global temperature rise is almost 1.1 °C higher than the value in the pre-industrial period (World Meteorological Organization 2017). In 2016, 195 countries ratified the Paris climate agreement including a commitment to keep the global warming below 2 °C before 2100. The global climate change is the result of a rise in Earth's temperature due to the presence of greenhouse gases from human activities. The limited availability of fossil resources, such as petroleum, and the strong dependence on the production of fuels and other chemicals provoke environmental, social, political, and economic concerns. Similar to the oil refinery, where the crude oil is processed and refined into different products of high and low values (liquefied petroleum gas, gasoline, naphtha for olefins and aromatics, kerosene, heating fuel, diesel, heavy fuel oil, and bitumen), the biorefinery is one of the most promising alternatives to obtain biofuels and chemicals from renewable sources (Chew et al. 2017). Biorefinery involves transformation of raw materials obtained from agriculture, silviculture, organic wastes, or any biomass through various unit processes to convert them in a wide range of products (Postma et al. 2016). Some definitions of biorefinery include the one provided by the National Renewable Energy Laboratory (NREL) "A biorefinery is a facility that integrates biomass conversion processes and equipment to produce fuels, power and (organic) chemicals from biomass" and by the International Energy Agency (IEA) "A biorefinery is the suitable processing of biomass into a spectrum of marketable products (food, feed, materials, and chemicals) and energy (fuels, power, heat)" (de Jong et al. 2012; Budzianowski 2017). Biorefineries are classified according to the biomass feedstock generation (Saai-Anuggraha et al. 2016; Hossain et al. 2017): The first-generation biorefineries use sugarcane, corn, or soybeans to produce value-added products for feed, food applications, fuels, and specialty chemicals. Almost all current biofuels (mainly ethanol, butanol, and biodiesel) and bio-based chemicals (lactic acid, itaconic acid, 1,3-propanediol, etc.) are produced in this type of biorefinery. The second-generation biorefineries are based on lignocellulosic materials, and they are composed of three main sections to convert lignocellulose into biofuels. The main product is the cellulosic ethanol; however, the biomass conversion by thermochemical platform involves the gasification of biomass to produce syngas (CO, CO_2, H_2, and CH_4), which can then be converted into various chemicals, such as ethanol, methanol, and butanol. The most advanced is the third-generation biorefinery that can use a mixture of biomass to produce a multitude of products using a combination of technologies. Microalgae biomass is considered as the most promising feedstock for the third-generation biorefineries. The microalgae might contribute to reduce the oil dependency and the rise in Earth's temperature. They have the ability to transform solar energy into chemicals by capturing CO_2 and releasing O_2. It is known that microalgae are one of the best technologies for carbon dioxide sequestration (Wiesberg et al. 2017) and their use as renewable energy source was long ago proposed by scientists. The patents and research papers indicate a strong interest in microalgae biorefinery looking for industrial-scale applications (Konur 2011; Mohan et al. 2016a, b; Xu and Boeing 2013; Zhu et al. 2016). The microalgae cultivation has high areal productivity, the possibility to grow in nonarable land, or wastewater used as nutrient source. From the biomass obtained, a spectrum of marketable products can be obtained, such as pigments, proteins, lipids, carbohydrates, vitamins, and antioxidants for applications like feed, food, polymers, pharmaceuticals, cosmetics, and biofuels (Borowitzka 2013; Budzianowski 2017; Suganya et al. 2016). Although microalgae biofuels are technically feasible, they remain strongly dependent on government subsidies and oil price, which make them economically nonviable for now (Wijffels and Barbosa 2010); therefore, primary strategies for bioenergy production from algae will need to rely on a multiproduct biorefinery approach (Laurens et al. 2017a). The CO_2 capture and the use of wastewater as nutrient source for microalgae growth combined with the production of high-value-added products and bioenergy make microalgae biorefinery potentially profitable.

2 Products Portfolio from Microalgae and Applications

The main goal of the biorefinery is to integrate the production of bioenergy (commodities: low-value high-volume products) and other chemicals (high-value low-volume products) to optimize the use of biomass resources by reducing wastes while maximizing profitability and benefits (Demibras 2009). Budzianowski (2017) categorized the high-value low-volume bioproducts from biorefineries into six groups: biopharmaceuticals, biocosmetics, bionutrients, biochemicals, biofertilizers, and biomaterials. All of them and biofuels can be obtained from microalgae (Chew et al. 2017; Milledge 2011). The microalgae products are reviewed in this section and summarized in Table 1 and Fig. 1.

Biopharmaceuticals: Microalgae are a source of many potential new drugs and bioactive molecules for health industry (Abd El Baky and El-Baroty 2013; Borowitzka 1995; Deniz et al. 2017; Mimouni et al. 2012). According to the number of patent publications, currently, biopharmaceutics is one of the most important innovation areas under development (Chilton et al. 2016). The bioactive molecules include applications, such as antioxidant, anti-inflammatory, antitumor, anticancer, antimicrobial, antiviral, and antiallergic agents along with other pharmaceutical properties (Deniz et al. 2017). Pigments, such as carotenoids (β-carotene and astaxanthin), phycobiliproteins (phycocyanin), and some polysaccharides or phenolic derivatives exhibit antioxidant and anti-inflammatory activities. Phycobilins have anti-inflammatory, antiallergic, antioxidant, and anticancer activities (Kim et al. 2016). Polyunsaturated fatty acids (PUFAs) are also of interest for human welfare, and there is a recent market of 11.5 billion dollars (Béligon et al. 2016). Molecules used for anticancer or antitumor effects include polysaccharides (carrageenan and fucoidan), PUFAs (eicosapentaenoic acid, EPA; or

Table 1 Microalgae portfolio adapted from Hamed (2016)

Application	Microalgae	Examples of functions and/or molecules	Market volume or price	References
Food and nutrition	*Spirulina platensis*, *Spirulina maxima*, *Chlorella*, *Muriellopsis* sp., *Crypthecodinium*, *Schizochytrium*	Food supplement. High content in proteins, iron, main unsaturated fatty acids, vitamin B12. Protection against hypertension and renal problems, support growth of lactobacillus (intestinal bacteria), immunostimulants, reduce free radical, effect on lipids levels in blood, can prevent gastric ulcers, wounds, and constipation. High levels of certain carotenoids to acting as antioxidant (like lutein) and used against degenerative diseases. PUFAs and EPA for infant formula	USD 12–50 millons per year for phycobiliproteins	Beheshtipour et al. (2013), Borowitzka (2013), Hamed (2016), Mata et al. (2010), Spolaore et al. (2006)
Feed	*Chlorella*, *Tetraselmis*, *Isochrysis*, *Pavlova*, *Phaeodactylum*, *Chaetoceros*, *Spirulina*, *Dunaliella*, *Skeletonema*, *Thalassiosira*	Used for their high nutritional value for zooplankton culture, fish farming, chicken breeding. As side effects, microalgae pigments can color flesh of salmon or shrimp exoskeleton and skin and other species. Gives additional value to aquaculture. It has positive impact on physiology for pets and farm animals and therefore on their appearance	USD 32 billions/year for *Chlorella* sp. (β-1,3-glucan, immune-stimulant in fish)	Brennan and Owende (2010), Das et al. (2012), Gouveia et al. (2008), Hemaiswarya et al. (2011), Mata et al. (2010), Priyadarshani and Sahu (2012), Sirakov et al. (2015), Spolaore et al. (2006)

(continued)

Table 1 (continued)

Application	Microalgae	Examples of functions and/or molecules	Market volume or price	References
Fertilizer and biostimulants	*Anabaena*, *Nostoc* *Spirulina*, *Haematococcus*, *Chlorella* sp., *Nannochloropsis* sp.	Upgrade soil quality and fertility by acting as water retainer, releasing essential nutrients (phosphate, nitrogen, and trace elements). Require less chemical fertilizer and act as promoters for plant growth and preventing plant infection by synthetizing biochemical components with antiviral and antibacterial activities	Biostimulant represents 27% of the USD 3.4 billons with prices of €1300–1500/ton for microalgal biostimulant	Abd El Baky and El-Baroty (2013), Hannon et al. (2010), van der Voort et al. (2015)
Cosmetic	*Spirulina*, *Chlorella*, *Haematococcus*	Proteins, vitamins, minerals, and pigments which have effects on skin. Skin-care and hair-care products already for sale. Help tissue to regenerate, collagen synthesis is improved	Astaxanthin produced by *Haematococcus pluvialis* has market prices between 1900 and 7000 USD/kg	Adarme-Vega et al. (2012), Hariskos et al. (2014), Shah et al. (2016), Spolaore et al. (2006), Yaakob et al. (2014)
Pharmaceuticals	*Nannochloropsis oculata*, *Tolypothrix byssoidea*, *Chlamydomonas*	Zeaxanthin pigment reduces tyrosinase activity by inhibition. Used in whitening creams. Tubercidin has activities in lymphocytic leukemia. Growth of lymphosarcoma (blood tumor cells) in mice has been stopped by L-asparaginase	Until today no microalgal-derived pharmaceuticals have entered the market yet. But total global pharmaceutical industry had a value of USD 955 billion in 2011 (LEK 2013), and the market of active ingredients for pharmaceuticals had a size of USD 101 billions	Ahmad et al. (2012), Paul (1982), van der Voort et al. (2015)

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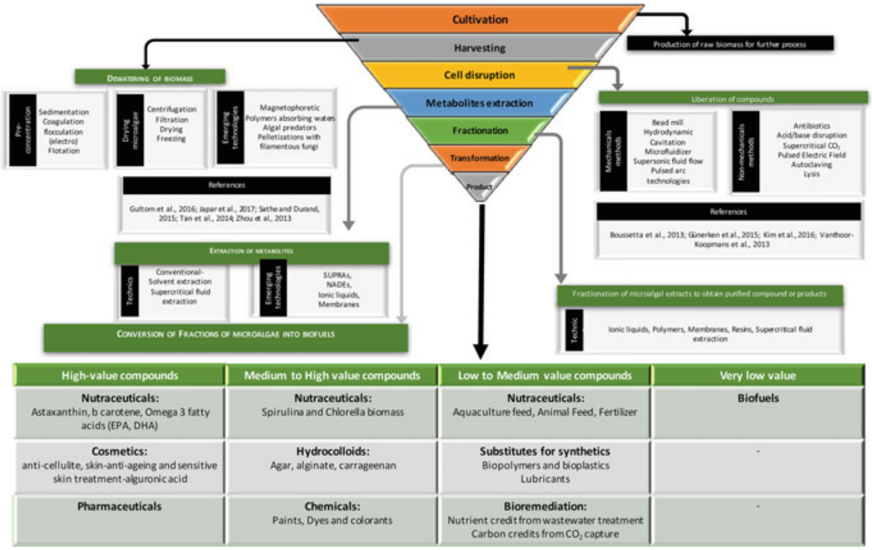
Table 1 (continued)

Application	Microalgae	Examples of functions and/or molecules	Market volume or price	References
Fuels and energy	*Scenedesmus almeriensis*, *Chlorella vulgaris*, *Chlamydomonas*, *Dunaliella*	Fatty acids for either biodiesel or other hydrocarbon fuels. Biogas from anaerobic digestion. Ethanol produced through fermentation	209,000,000 ton for fatty acids at 920 USD/ton	Chew et al. (2017), Chilton et al. (2016), Laurens et al. (2017a)
Materials	*Chlorella infusionum*, *Dunaliella*, *Schizochytrium*	Starch and proteins for a wide range of bioplastics. Phytol for and sterols for surfactants. Amino acids or peptides for either polyurethane and plasticizers	1.5 millons ton for thermoplastics from protein at 1900 USD/ton	Laurens et al. (2017a)
Chemical	*Dunaliella*, *Schizochytrium*	Lactic acid, biomethanol, glutamic acid, sorbitol, glycerol for chemical industries	36,000–2,300,000 ton for glycerol at 1550–3400 USD/ton	Bozell and Petersen(2010, Budzianowski (2017), Laurens et al. (2017a)



High-value compounds	Medium to High value compounds	Low to Medium value compounds	Very low value
Nutraceuticals: Astaxanthin, b carotene, Omega 3 fatty acids (EPA, DHA)	**Nutraceuticals:** Spirulina and Chlorella biomass	**Nutraceuticals:** Aquaculture feed, Animal Feed, Fertilizer	**Biofuels**
Cosmetics: anti-cellulite, skin-anti-ageing and sensitive skin treatment-algluronic acid	**Hydrocolloids:** Agar, alginate, carrageenan	**Substitutes for synthetics** Biopolymers and bioplastics Lubricants	-
Pharmaceuticals	**Chemicals:** Paints, Dyes and colorants	**Bioremediation:** Nutrient credit from wastewater treatment Carbon credits from CO_2 capture	-

Fig. 1 Downstream processing in a biorefinery

docosahexaenoic acid, DHA) and phycobiliproteins (phycocyanin). Antimicrobial and antiviral applications are related to fatty acids, pigments, peptides, and polyphenols.

Biocosmetics: Microalgae components are often used in cosmetics as water-binding agents, texturizing agents, antioxidants providing dermal protection as well as skin-whitening agents (Jahan et al. 2017; Stolz and Obermayer 2005; Wang et al. 2015). Compounds such as sporopollenin, mycosporine-like amino acids, and scytonemin protect against UV-A or UV-B radiation. Carotenoids are also used as stabilizers in cosmetics and solar protection products. Phycocyanin, a natural blue pigment, is extensively used in cosmetics, including lipsticks, eyeliners, nail polishes, and eye shadows. Red-phycoerythrin is used as an alternative for synthetic pigments in creams or other cosmetics. Collagen-like proteins are included in creams and gels with high moisturizing action, but their other activities are also known, including antiaging and antiwrinkling. Some polysaccharides, such as chitin or fucoidan, have protective and moisturizing properties. Other polysaccharides, such as agar, carrageenan, and alginates, are used as stabilizers, thickeners, and emulsifiers. Skin-whitening and antimicrobial agents are related mostly to compounds synthetized by macroalgae; however, microalgae extracts have exhibited antimicrobial effects too (Martins et al. 2008). Based on patent landscape, this sector is dominated by European countries and, specifically, by France (Chilton et al. 2016).

Bionutrients: Microalgal biomass is a promising source of nutrients. Indeed, both food and feed sectors have been quickly increasing in recent years to replace animal protein. Nourishment is the fourth area of applications of microalgae feedstock, and based on patent landscape, human nutrition is the second most important sector (Chilton et al. 2016). Aquaculture is a special case with an average growth (35%) much higher than other areas (20%) (Chilton et al. 2016). Dry biomass powder with high nutrient content and valuable compounds included, such as fatty acids, pigments, and antioxidants, is the main product presentation (Hamed 2016). Proteins, carbohydrates, lipids, and vitamins are of great interest for nutrition as well as pigments like: yellow-orange carotenes and xanthophylls, the red or blue phycobilins, and green chlorophylls. They have applications as natural colorants for food industry or supplements for both human and animal nutrition. High-quality proteins are produced by microalgae like *Spirulina* (Becker 2007) and *Chlorella*, which are identified as "super food" (Milledge 2011) and commercialized as nutraceuticals. Microalgae food is mainly commercialized as dried algae (*Chlorella* and *Spirulina*) and sold as dietary supplements or found as specialty products, extracted/isolated from the microalgae and added to food/feed to improve their nutritional value (pigments, antioxidants, proteins, and fatty acids, e.g., omega-3, DHA, and EPA). The market size of nutrients obtained from microalgae is still significantly smaller in comparison with the one derived from crops, but this sector has an impressive and unique growth (Vigani et al. 2015).

Biochemicals: Market projection predicts that 17–38% of total organic chemicals will be provided by biochemicals around 2050 (Budzianowski 2017). The US Department of Energy (DOE) registered ten biochemicals with high future potential for the market (Bozell and Petersen 2010): biohydrocarbons, succinic acid, furanic, glycerol and derivatives, lactic acid, levulinic acid, hydroxypropionic acid/aldehyde, xylitol, sorbitol, and ethanol. Numerous biochemicals, such as biomethanol, lactic acid, glutamic acid, sorbitol, glycerol, and 3-hydroxypicolinic acid (3-HPA), are already used in industries like BioMCN or Roquette Freres SA (Broeren et al. 2013). Further reduction of production costs will allow expanding their applications. Other products, such as alginates, xylose, or glucaric acid, are however unique, and their specific market does not exist yet (Budzianowski 2017). Microalgae produce various building blocks for biochemicals, and these are the largest class of high-value bioproducts that could be obtained in a biorefinery, such as pigments and PUFAs (Budzianowski 2017).

Biofertilizers: They have great potential to replace chemical fertilizers and avoid the aggressive use of chemicals that leads to soil erosion and degradation of local ecosystems through eutrophication when they run off into rivers or percolate into groundwater. Likewise, their use contributes indirectly toward greenhouse gas emissions as their production depends on fossil fuels. Biofertilizers include the nitrogen-fixing, phosphate solubilizing, and plant growth-promoting microorganisms. Microalgae have important role in soil ecosystems (Pulz and Gross 2004). According to Chatterjee et al. (2017), microalgae contribute to soil fertilization through: (1) enhancement of soil porosity because of the filamentous structure and production of adhesive substances of certain cyanobacteria; (2) release of growth promoters, such as amino acids, hormones (auxins, gibberellins, cytokinins), and vitamins (Pulz and Gross 2004; Singh et al. 2016); (3) increase in water retention capacity through their thickened structure (Hamed 2016); (4) soil enrichment with organic matter and minerals after death and decomposition of microalgae biomass (Saadatnia and Riahi 2009); (5) reduction in soil salinity (Al-sherif et al. 2015); (6) prevention of weed growth and production of antiviral and antibacterial substances to protect plants (Abd El Baky and El-Baroty 2013; Dahms et al. 2006; Hannon et al. 2010); and (7) increase in soil phosphate by excretion of organic acids (Singh et al. 2016). Some nitrogen-fixing species, such as *Anabaena* and *Nostoc*, can be directly used as fertilizers for agricultural purposes (Hamed 2016) through direct inoculation in soils, or green algae can be applied as dry powder with high percentage of macronutrients, considerable amounts of micronutrients, and amino acids (Faheed and Abd-El Fattah 2008; Garcia-Gonzalez and Sommerfeld 2016).

Biomaterials: Biomaterials use complex structures of biomass for application in plastics, coatings and surface treatment materials, packaging materials, fibers and textiles, elastomers, lubricants and fillers, surfactants, and functional materials (Budzianowski 2017). Biomaterials have a bright future in replacing materials from fossil resources. The biochemical composition of biomass defines the potential biomaterial that can be produced. Proteins are the main platform molecules to make thermoplastics, foams, adhesives, biocomposites, and flocculants, and bioplastics are made from starch (Laurens et al. 2017a). In the case of microalgae biomass, bioplastics can be derived from any of the three major component fractions (lipids, proteins, and carbohydrates) (IEA 2017). Some researchers have described the use of the whole algae as filler material for different types of plastics, such as polypropylene (Zhang et al. 2000a), polyvinyl chloride (Zhang et al. 2000b, polyethylene (Otsuki and Zhang 2004; Zeller et al. 2013), blends of algae and starch (Kipngetich and Hillary 2013), or proteins (Reddy et al. 2013; Shi et al. 2011). But microalgae can also produce high-quality biodegradable plastics, such as polyhydroxyalkanoates (PHA) (Balaji et al. 2013; Chaogang et al. 2010; Haase et al. 2011; Rahman and Miller 2017). Surfactants can also be produced from microalgal sterols and phytol and have a high market potential of around 8,436 billion dollars for a five-year period (IEA 2017; Laurens et al. 2017a). Furthermore, asphalts can be made from microalgae biomass as well (Chailleux et al. 2012).

Bioenergies: A wide range of biofuels for bioenergy can be produced from microalgae biomass and all petroleum fuels, such as hydrocarbons, asphalts, liquid (kerosene, gasoline, diesel), and gaseous fuels (methane, syngas); even more, the biocrude can be made from microalgae biomass (Bahadar and Bilal Khan 2013; Budzianowski 2017; Chew et al. 2017). Biofuels are the third sector in terms of patent applications due to decades of research. However, given the noneconomic viability, this area has experienced a slow growth although it has aroused a lot of interest (Chilton et al. 2016). Hydrogen can be produced directly by microalgae photolysis. Other biofuels, such as ethanol and biogas, can be obtained from transformation of carbohydrates (starch, sugars, or other polymers) by fermentation into bioethanol (Chng et al. 2015) and/or anaerobic digestion, respectively. Bioelectricity can be also generated by integration of microalgae into a microbial fuel cell using microalgae in the cathode compartment and bacteria in the anode (Gouveia et al. 2014; Lee et al. 2015). This integration becomes especially favorable when considering that phototrophic organisms act as in situ generators of oxygen facilitating the reaction in the cathode of the chamber. Bioelectricity is produced by bacteria in the anode, which oxidize organic matter and produce electrons. Those electrons are transferred to the cathode electrode with an external circuit and produce electricity. The bacteria can be used for biodegradable waste treatment, and with the help of microalgae, the organic and inorganic load of the water can be reduced.

From all the above-mentioned bioproducts, the role of microalgae in the human diet is well established, but other applications are currently under development: biofuel production of pharmaceutical compounds, bioremediation, cosmetic active ingredients. Furthermore, microalgae produce many environmental benefits, such as carbon fixation, oxygen release, heavy metal removal, and wastewater treatment that provide energy savings and supply oxygen to anaerobic bacteria (Uggetti and Puigagut 2016). However, market is clearly dependent on actual investigation of new technologies, and mainly on governmental policies such as subsidies and mandated use of biofuels (Gorry et al. 2017).

3 Microalgal Biomass Processing

In order to maximize the potential of microalgae biomass, the whole chain process development should be defined in an integrated way, starting from an adequate supply of nutrients and CO_2, good harvesting methods, dewatering, and downstream processing (Mata et al. 2010; Toledo-Cervantes and Morales 2014). For this, it is necessary to know not only the potential added value that can be obtained, the microalgae cell wall strength and the composition and localization of cellular components in order to break down the cell wall properly to avoid product loss (Gerardo et al. 2015; Pei et al. 2010; Roux et al. 2017), but also the available processing technologies and the sequence of separation; these latter ones are needed to maintain the integrity of the possible products maximizing the recovery and to produce biofuels. Each biorefinery stage for processing microalgal biomass would be linked to the characteristics of each specific strain and biochemical composition, and the route to obtain bioenergy must be defined too. Main downstream processing technologies are explained in the following sections (see Fig. 1), and the routes to obtain diverse biofuels are shown as well.

3.1 Downstream Processing

3.1.1 Harvesting Technologies

Harvesting accounts for 20–30% of microalgae biomass production cost that is associated with the recovery of microalgae biomass from diluted streams (Barros et al. 2015; Pei et al. 2010; Tan et al. 2014). Harvesting is an energy-intensive process; therefore, it is necessary to choose an effective procedure to concentrate the biomass with low energy to minimize separation costs. The main technologies and emerging options for microalgae recovery are shown in Fig. 1 and Table 2. Harvesting includes physical (centrifugation, sedimentation, and filtration), chemical (flocculation, flotation, auto-flocculation, and bioflocculation), and electric (electro-coagulation–filtration or electrochemical harvesting) alternatives. Japar et al. (2017) established that filtration, flocculation, bioflocculation, and electro-coagulation-filtration and further drying using solar heat to process the algal cake are the most feasible solutions to remove water due to their high harvesting efficiencies, moderate operational and logistic costs, no negative impacts on the environment, and the shortest harvesting time. However, in a biorefinery, the combination of separation processes is recommended: a first step where the biomass is concentrated with a mechanical or chemical process to obtain a final concentration around 2–7% of total suspended solids, and a second, dewatering step to produce a microalgal cake (Barros et al. 2015; Gerardo et al. 2015). The pre-concentration step reduces the energy necessary to separate biomass from water. However, the dewatering process must be established depending on the strain and the final product requirements, so more research is required to reduce the energy requirement and lower microalgal harvesting costs (Barros et al. 2015). New emerging technologies include ultrasound, magnetophoretic procedures, the use of polymers to absorb water, and co-culture with fungi to form flocs (Xia et al. 2011; Zhou et al. 2013) that favor the removal of solids; however, additional research is still required.

3.1.2 Cell Disruption

Microalgae store most of their valuable components inside the cell, behind a thick and resistant cell wall. Therefore, energy - or solvent- consuming steps are needed to alter this physical barrier and to efficiently extract the desired compounds. A mild cell disruption method is necessary to make cell components available without losses. Cell disruption technologies can be divided into two main categories: mechanical and nonmechanical methods (see Fig. 1). Mechanical methods include: bead milling, homogenization, sonication, microwaving, thermolysis, freezing, use of chemicals, electroporation, supersonic flow, among others. Detailed information about principles, advantages, and disadvantages can be found elsewhere (Halim et al. 2012b; Günerken et al. 2015; Postma et al. 2016; Toledo-Cervantes and Morales 2014). The cell disruption method depends on the cell wall characteristics (Eppink et al. 2017) and must be carefully selected because some cell components can be denatured (Günerken et al. 2015; Pei et al. 2010). The cell wall is a barrier that separates the cellular content from the surrounding aqueous medium. Its composition is strain-dependent, but is usually composed of polysaccharides (cellulose, hemicellulose, etc.), lipids, and membrane proteins, which can adopt different structures (Baudelet et al. 2017). For instance, Chlorella has two or three layers with different structures, such as a transparent microfibrillar layer and

Table 2 Harvesting technologies, adapted from Barros et al. (2015) and Gerardo et al. (2015)

	Technology	Function	Advantages	Difficulties
Primary step: pre-concentration	Sedimentation	Based on gravity for the cell to freely settle	No energy needed Very low cost	Slow process that can affect biomass, relatively high superficies required to treat high volume Final concentration still very low
	Coagulation–flocculation (electro)	Use of a chemical or bioflocculants that use the electrostatic properties of a microalgae cell to make it aggregate in larger flocs to enhance settling velocity	Simple, better, and faster sedimentation as microalgae forms aggregates No energy use if not electro-coagulation	Use of additives which can be expensive and toxic And/or high additional energy for electro-coagulation Recycling of culture medium is limited
	Flotation	Gas vesicles are used to help microalgae going upward and float near the surface; a surfactant can be aggregated	Low cost Low space required Short operation times	Use of additives to make flocculates Inapplicable in case of marine microalgae
Secondary step: biomass recovery or dewatering	Centrifugation	Based on centrifugal force	High efficiency Fast Almost any microalgae can be centrifuge	Expensive as it requires high quantity of energy Viable only for recovery of high-value products Possible cell damage
	Filtration	Filtration with membranes	High efficiency Free of any additive chemical hazardous	Fouling/clogging which increases operational cost Membranes must be cleaned regularly Relatively high cost due to pumping and membrane replacement

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Table 2 (continued)

	Technology	Function	Advantages	Difficulties
Emerging technologies	Ultrasound	Ultrasound is used to exploit dielectric properties of microalgae cells by forcing them to move to the nodules of the standing waves, flocculate, and sediment	High efficiency Potential for multipurpose use as: cell lysing with lower frequencies and higher pressures	High energy cost compared to other technologies including filtration and centrifugation
	Magnetophoretic (Japar et al. 2017)	Use of magnetophoretic properties of microalgae by adding a magnetic nanoparticle which will link microalgae cells into flocculates and be removed	Easily remove from broth High efficiency of recovering biomass High efficiency of recycling magnetic particle and can be reused	Energy intensive due to need for agitation and use of compressors and shear mills High efficiency can be maintained only for low flow rates (<0.6 L/h)
	Polymers absorbing water (Japar et al. 2017)	Super-absorbent polymers which will immobilize biomass	Fast High concentration factor	Separation of biomass for polymers still unclear
	Algal predators (bacteria) (Sathe and Durand 2015)	Flocculating agent	Potent flocculating agent Inexpensive Eco-friendly	More investigation necessary
	Attached microalgal growth	High velocity culture in PBR have a support inside the culture medium where microalgae can fix itself	Easy recovery	Depend on the microalgae ability to form film
	Pelletizations with filamentous fungi (Gultom et al. 2014; Tan et al. 2014, Xia et al. 2011)	Fungi will trap microalgae cell in their filamentous and make pellets of high weight which make them sediment	Low cost of culture (wastewater, sucrose) (Zhou et al. 2013)	2 days to sediment Need to be further filtrated

trilaminated structure (Yamada and Sakaguchi 1982); hence, the cell disruption of Chlorophyta is hard because they have rigid and thick cell walls (Baudelet et al. 2017). Additionally, culture conditions could alter the cell wall structure and composition (Eppink et al. 2017).

According to Parniakov et al. (2015), the pulse electric field (PEF) seems promising for a controlled cell wall disruption as pre-treatment or combined with other treatment processes, such as sonication or extraction with a green solvent (Postma et al. 2016). But this technology has some disadvantages; e.g., the solution must be free of ions, and energy consumption is strongly dependent on biomass concentration (Günerken et al. 2015). Nevertheless, more research is needed to improve the efficiency of the cell wall disruption for different microalgae biomass concentrations and the liberation of products needs to be increased. Electrical arc treatment is a relatively recent technique for extraction from biomass. This technique was applied for polyphenol extraction and resulted in lower energy consumption, 16 kJ/kg compared to 53–267 kJ/kg for PEF (Boussetta et al. 2013). This could be of high interest for microalgae biorefinery. Two other processes are promising: the subcritical water use and the high-pressure homogenization (Roux et al. 2017), which suggest a positive energy balance for cell disruption.

3.1.3 Metabolite Extraction

After cell disruption, the next step is the extraction of products. Extraction methods include the use of solvents, super- or subcritical fluids, polymers, ionic liquids, membranes, or resins (see Table 3). The main objective of extraction is to obtain all fractions with no loss either in quantity or in quality (avoiding alteration/loss in functions). Reviews about this topic have been done by Eppink et al. (2017), Gong et al. (2017), González-Delgado and Kafarov (2011), Michalak and Chojnacka (2014), Postma et al. (2016), Roux et al. (2017), and improvements have mainly focused on fuel/lipid extraction by either solvent or supercritical fluid extraction. Conventional extraction procedures for lipids are hydraulic pressing, expeller pressing, and solvent extraction (Cuellar-Bermudez et al. 2015; Ranjith Kumar et al. 2015). For solvent extraction, hexane, hexane-isopropanol, or chloroform-methanol are the main solvents used (Cuellar-Bermudez et al. 2015; Ranjith Kumar et al. 2015). The adequate solvent blend must be chosen depending on lipid polarity and solubility (Cuellar-Bermudez et al. 2015). The ideal solvent blend for lipid extraction from microalgae seems to be chloroform-methanol in a 1:1 (%v/v) proportion (Ryckebosch et al. 2012). A wet technology for lipid extraction was studied by the NAABB (National Alliance for Advanced Biofuels and Bioproducts) at laboratory-scale and showed good performance with selective separation of free fatty acids and tocopherol; this alternative offers energy savings because harvesting and drying operations are not necessary (Marrone et al. 2017). Recent advances have also been made in supercritical fluid extraction (SFE) (Nobre et al. 2013; Yen et al. 2015). One advantage of SFE is the application for extraction of both lipids and pigments. Nobre et al. (2013) achieved $33\text{g}_{\text{lipid}}/100\ \text{g}_{\text{dry biomass}}$

Table 3 Extraction technics

Extraction techniques	Advantages	Disadvantage	References
Water-soluble techniques			
Autoclaving (subcritical water)	High efficiency, solvent eco-friendly, recyclable, nontoxic solvent, direct process from culture with concentration step, subcritical water	High energy consumption, not suitable for delicate compounds sensible to temperature	Gong et al. (2017)
Boiling	Quick, solvent eco-friendly, recyclable, nontoxic solvent, direct process from culture with concentration step, good to extract phenols	High energy consumption, not suitable for delicate compounds sensible to temperature, low efficiency	Godlewska et al. (2016)
Homogenization (high pressure)	High efficiency, co-extraction, can be eco-friendly and nontoxic (use of green solvent)	High energy consumption not adequate for compounds sensible to temperature and/or pressure	Mulchandani et al. (2015)
Novel extraction techniques			
Supercritical fluid extraction (SFE)	High efficiency, cheap, high removal rate, eco-friendly, with CO_2 for thermolabile molecules and other fragile compounds, recyclable	High inversion, supercritical CO_2 nonpolar	Cuellar-Bermudez et al. (2015), Grosso et al. (2015), Michalak and Chojnacka, (2014), Nobre et al. (2013), Ranjith Kumar et al. (2015), Taher et al. (2014), Yen et al. (2015)
Ultrasound-assisted extraction (UAE)	Reduce quantity of solvents used, high performance and process faster due to quicker kinetics, equipment cheaper than other novel techniques, scalable, combine to MAE and SFE possible, reaction and extraction can be joint	Increase in energy consumption	Michalak and Chojnacka (2014)

(continued)

Table 3 (continued)

Extraction techniques	Advantages	Disadvantage	References
Microwave-assisted extraction (MAE)	Reduce quantity of solvents used, high performance and process faster, reaction and extraction can be joint	Increase in energy consumption, a further step is needed to split solid residue from liquid phase	Michalak and Chojnacka (2014)
Pressurized liquid extraction (PLE)	Reduce quantity of solvents used, faster than other solvent extraction, shorter time of process, an extended variety of solvents can be occupied for PLE, more compliant for bioactive molecules than SFE	Cannot be used for bioactive compounds susceptible to temperature due to high temperature and pressure of the process	Michalak and Chojnacka (2014)
Enzyme-assisted extraction (EAE)	Bioproducts easily freed, eco-friendly, nontoxic, no increase in energy consumption, easy separation of molecules, scalable	High cost of enzymes, enzyme selectivity which will increase cost to make an efficient cocktail, yield depending on enzyme selectivity	Michalak and Chojnacka (2014)
NADESs	Eco-friendly, biodegradable, nontoxic, low cost compared to DESs Made of primary metabolites such as amino acids, organic acids, sugars, and choline derivatives Used for phenols and flavonoids extraction	Further study of lipids extraction with theses solvents needs to be done	Espino et al. (2016), Jeevan Kumar et al. (2017)
SUPRASs	Nanostructured amphiphile liquids, solvent improvability is high, appropriate for extraction as existence of various polarity areas	Efficiency needs to be demonstrated for extraction	Ballesteros-Gómez et al. (2010), Jeevan Kumar et al. (2017)

(continued)

Table 3 (continued)

Extraction techniques	Advantages	Disadvantage	References
Deep eutectic solvents (DESs)	Safe, cheap, multi eutectic fluid system (two or more solvents), biodegradable, nontoxic, production of such solvents is inexpensive, extensive polarity	Promising results where demonstrated, application area needs to be defined	Jeevan Kumar et al. (2017), Jeong et al. (2015), Paiva et al. (2014)
Fluorous solvents	Nontoxic, hydrophobic and lipophobic, inert in nature, employed for trace metals extraction and fractionation of oils, phase easily split up	Lipid extraction through fluorous solvents needs to be studied	Horváth (1998), Jeevan Kumar et al. (2017)
Acid and alkaline hydrolysis	High efficiency, fast	Use of high concentration of acids or alkaline	Dong et al. (2016), Jeevan Kumar et al. (2017), Roux et al. (2017)
Conventional solvent extraction			
Extraction in Soxhlet apparatus	High efficiency, easy to scale up thanks to its simple operating system, safety	Toxicity	Halim et al. (2012a), Michalak and Chojnacka (2014)
Solid–liquid extraction (SLE)	Easy to scale up	Toxicity	Michalak and Chojnacka (2014)
Liquid–liquid extraction (LLE)	Easy to scale up	Toxicity	Michalak and Chojnacka (2014)

from *Nannochloropsis* sp. biomass when supercritical CO_2 was used, and they observed an increase of 36% when ethanol was added as co-solvent; in this case, the global recovery was around 85% for lipids, and 70% of pigments. Ethanol allows faster extraction and is suitable for feed and nutraceutical applications. The main advantage of SFE with CO_2 is the use of a nontoxic, cheap, safe, and chemically inert solvent at adequate critical temperature and pressure. Water is another good candidate for SFE being nontoxic and cheap, but high pressure and temperature necessary to reach its critical point involve higher energy costs than SFE with CO_2.

On the other hand, innovative solvent-free methods such as osmotic pressure or isotonic (also called ionic) solvent have started to be investigated (Ranjith Kumar et al. 2015). They are eco-friendly, since they avoid the use of toxic solvents, have lower energy consumption, and are considered cheaper alternatives (Adam et al. 2012). Ionic liquids, nonaqueous salt solutions that comprise an organic cation and a polyatomic inorganic anion, are becoming popular and high extraction yields are expected due to their chemical nature. They are considered as green solvents (Eppink et al. 2017; Halim et al. 2012a; Kumar et al. 2016), because they reduce energy consumption, allow the use of alternative solvents and renewable natural products, and ensure a safe and high-quality extract/product" (Chemat et al. 2012).

Ionic liquids are nonvolatile, thermally stable, and also have the capacity to disturb cells and destabilize them (Park et al. 2015). Grosso et al. (2015) suggest the use of switchable solvents to improve the extraction, using an alcohol and an amine base in a nonionic state that after injection of CO_2 turn into an ionic liquid; finally, to recycle the solvent, N_2 is injected through the solvent turning it back to nonionic state. There is another kind of switchable solvents, such as hydrophilic solvents (Boyd et al. 2012; Jessop et al. 2012). Some interesting reviews about green extraction were made by Du et al. (2015) and Jeevan Kumar et al. (2017). Green techniques allow a lower use of solvent, improve product quality, do not affect other biocompounds, and, moreover, induce a decrease in energy consumption (Jeevan Kumar et al. 2017). Emerging green solvents include natural or deep eutectic solvents (NADESs) and supramolecular solvents (SUPRASs). NADESs play a role as alternative media to water in living organisms. The main reason to use this other medium is to help survival of any organism under harsh conditions, such as cold or dryness, and therefore, NADESs are mostly made up of sugars, urea, choline chloride, and organic acids (Jeevan Kumar et al. 2017). SUPRASs are nanostructured liquids that consist of assemblies of amphiphiles dispersed in a continuous phase.

Novel approaches consist in combining a disruption method with an extraction method to enhance the global process and make it greener as is the case with microwave-assisted extraction (MAE) or ultrasound-assisted extraction (UAE), enzyme-assisted extraction (EAE), pressurized liquid extraction (PLE) combined with solvent extraction or other techniques (Ibañez et al. 2012; Kadam et al. 2013).

Regarding protein extraction, fragile proteins are of economic interest and extraction of the protein fraction after cell lysis using mild technologies is incipient (Eppink et al. 2017). Proteins are mainly recovered with solvents by filtration (micro- and ultrafiltration) (Marrone et al. 2017) or through precipitation by pH shifting (Ursu et al. 2014). Extraction of proteins through tangential ultrafiltration and neutral pH has a relatively high yield without alteration of protein functionality (Ursu et al. 2014). Filtration requires little energy and is considered a green and mild process because it does not change protein state compared to extraction with solvents (Safi et al. 2017). However, precipitation is considered a better option to obtain protein powder and also to reduce the operating costs (Ursu et al. 2014). A recent interest has emerged for the use of polymers within the aqueous two-phase system (ATPS) looking for mild separation and extraction of proteins. This system is prepared using a polymer–polymer and a polymer–salt mixture in such a way that two water-rich phases are formed, thus providing the necessary gentle solvent for proteins that does not affect their functionality. Zhao et al. (2014) proposed a multiple stage ATPS extraction in order to increase purity of C-phycocyanin from *Spirulina platensis*. Phong et al. (2017a) combined UAE and ATPS for protein recovery of *Chlorella sorokiniana* finding that phases could be recycled at least five times.

On the other hand, high-value pigments are commonly extracted using solvents or supercritical fluids. Enhancement of pigment extraction has also been investigated through combination of solvent extraction with other methods, such as ultrasound or microwaves (Halim et al. 2010; Pasquet et al. 2011).

Lastly, classical solvent extraction can be used for carbohydrate recovery but improvements in recovery have been reported using fluidized bed extraction or ultrasonic-assisted extraction. However, this enhancement was associated with higher operating costs (Zhao et al. 2013). Wu et al. (2017) proposed a high-speed counter current chromatography (HSCCC) combined with ATPS extraction to recover high-purity polysaccharides in a single-step extraction process. Carbohydrates from microalgae have aroused recent interest in the biorefinery process (IEA 2017; Templeton et al. 2012).

Emergent technologies for protein recovery include the liquid biphasic flotation based on the combination of ATPS and solvent sublation. This technique allows the integration of concentration, separation, and extraction into one step, along with a higher concentration coefficient (Phong et al. 2017a, b).

3.1.4 Fractionation

Fractionation could be required in purification train after extraction and depending on the application of microalgae products. It focuses on the primary recovery and partial purification of products with no loss in products and functionality. The goals of fractionating microalgae biomass are either to separate lipids, proteins, and carbohydrates for further valorization of each fraction or to obtain a specific compound. Hence, the microalgal extracts from either hydrophobic and hydrophilic phase can be separated using common techniques based on density differences and further selective techniques (see Table 4), such as ionic exchange chromatography, charged membranes or protein precipitation (Schwenzfeier et al. 2011, 2014) allow isolation of proteins from a common hydrophilic phase where carbohydrates are also present. On the other hand, complex high-cost downstream processing is used when isolation of a specific compound, such as PUFAs, from lipid fraction (Dibenedetto et al. 2016) or high-grade protein is required (Halim et al. 2016). Therefore, developments in fractionation are still limited for high-value products due to its high cost and feasibility is only attainable in domains, such as food, health, and cosmetics. Indeed, this is an incipient area that needs development but thanks to biopharmaceutical field, mild extraction techniques are being adopted for microalgae specialty products.

Membrane technologies are commonly used for biomass harvesting (Drexler and Yeh 2014). They provide a thin barrier to restrict the interactions between the solvent and solute depending on their properties and membrane characteristics; however, finest filtration methods, such as microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO), allow selective product separation.

Table 4 Fractionation technics applications and advantages

	Methods	Applications and other advantages
Pre-fractioning		
Polymers (Cuellar-Bermudez et al. 2015; Grosso et al. 2015)	Aqueous two-phase system (ATPS) separation: mix of a polymer–polymer, polymer–salt beyond a critical concentration will form two phases	For protein extraction and purification Alternative to chromatography
Ionic liquids (Grosso et al. 2015; Suarez Ruiz et al. 2017)	Simple molten salts in forms of cations and anions, separate in hydrophilic and hydrophobic phase	Applications for organic synthesis, liquid-phase extraction, and catalysis for clean technology and separations Able for recycling with minimum pollution compared to organic solvents Separation of hydrophilic (e.g., carbohydrates, proteins) and hydrophobic (e.g., pigments, lipids) Novel approach: mix of organic solvents (e.g., ethyl acetate) and ionic liquids
Fractionation (Pei et al. 2010; Ranjith Kumar et al. 2015; Taher et al. 2014)		
Membranes (Demmer et al. 2005; Gerardo et al. 2014; Marcati et al. 2014; Safi et al. 2014b; Schwenzfeier et al. 2014, 2011; Van Reis and Zydney 2001)	Separation of carbohydrates and proteins and pigments traces	Tangential flow presents the advantages of fractionation with different filter sizes, from 1 to 1000 kDa, under mild conditions New developments of filter: dead-end filtration with a layer of specific ligands (charged, hydrophobic, hydrophilic) with the objective to extract a precise protein component; further fractionation is possible
Resins (Bermejo et al. 2006; Cuellar-Bermudez et al. 2015; Schwenzfeier et al. 2014)	Chromatographic separation (size exclusion or ionic exchange)	Protein mixture was fractionated with ionic exchange chromatography. Technology mainly used for high-value products in food/health/cosmetics
Extraction (Cuellar-Bermudez et al. 2015; Gilbert-López et al. 2015; Grosso et al. 2015; Taher et al. 2014)	Solvent extraction or supercritical fluids Nanofiltration	Recent research points out the supercritical fluids as a promising technology for scalable extraction of pigments and lipids

Residual pigments and carbohydrates are separated from the hydrophilic phase through dead-end or tangential flow membrane filtration (Gerardo et al. 2014; Lorente et al. 2017; Marcati et al. 2014; Safi et al. 2014a, b; Schwenzfeier et al. 2011, 2014; Van Reis and Zydney 2001). Besides, membrane technologies can be combined with other processes to increase selectivity by combining principles of other fields (Demmer et al. 2005) for isolation of specific proteins using adsorbent particles embedded in membrane pores or selective aqueous buffer systems for the next fractioning step of carbohydrates and/or proteins (Weaver et al. 2013).

High-resolution chromatography has also been used for fractioning product recovery. In a first step, Schwenzfeier et al. (2011) characterized *Tetraselmis* sp. fractioning with a mild process and ionic exchange chromatography was used to obtain protein. High purity can be also reached through ionic exchange chromatography and size exclusion chromatography for phycoerythrin from *Porphyridium cruentum* (Bermejo et al. 2006; Cuellar-Bermudez et al. 2015). Those highly purified proteins could be of interest for clinical and pharmacological research as they can present some properties interesting for health, such as antioxidant or anticancer activities.

3.1.5 Selective Extraction

Some techniques, such as ionic liquids, SFE, and ATPS, are applied to hydrophobic phase for further separation of their different compounds (PUFAs, glycolipids, phospholipids) from oily fraction. Solvent extraction or SFE is specifically used to split up lipids and pigments (Cuellar-Bermudez et al. 2015; Grosso et al. 2015). Innovative processes, such as direct transesterification during SFE, are commented by Ranjith Kumar et al. (2015) and Taher et al. (2014) in reviews, but they need deeper research for scaling up to industrial scale.

3.2 Processing Biomass to Obtain Energy

Processes involved in microalgae biomass transformation in the biofuel-driven biorefineries are classified into direct combustion, thermochemical or biochemical processing, and chemical transformation (see Fig. 2) involving the chemical transformation of lipids extracted from biomass to produce biodiesel through transesterification. All of them are explained in the following sections.

3.2.1 Direct Combustion

It is the most direct route to utilize microalgae biomass as fuel. Direct combustion is a thermochemical technique used to burn biomass in the presence of excess air. In theory, algae can be dried and burned. Combustion of algae for power generation

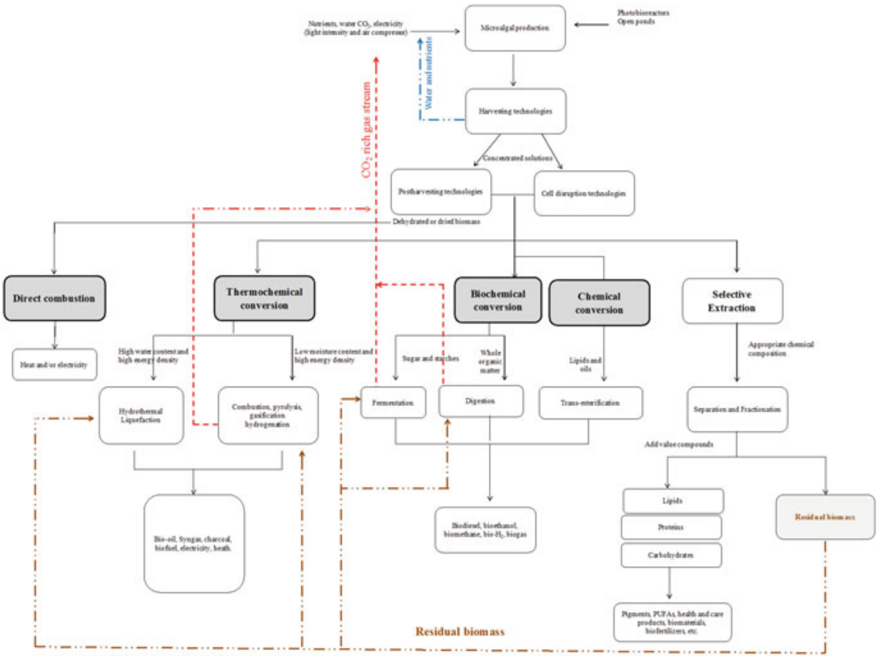

Fig. 2 Thermochemical and biochemical processing in a microalgae biorefinery for production of biofuels and value-added compounds (adapted from Toledo-Cervantes and Morales 2014). Brown dotted lines mean the incorporation of residual biomass to produce biofuels, blue dotted lines indicate water recycling, and red dotted lines are CO_2 gas stream generated during microalgae biofuel processing and reincorporated for biomass growth

has never been attempted on a large scale, in part because the large-scale cultivation operations have focused on other, more economical uses of algae. Drying is not difficult, and several methods have been standardized, including sun-drying, drum drying, vacuum drying, and freeze-drying. These methods are capable of reducing the moisture content to ~2%. Because it is not possible to burn biomass directly in an internal combustion engine, the technology for power generation could be a Rankine engine using a forced convection industrial boiler. A system with this configuration would require an area of ~20 × 20 m and a continuous power output of a kilowatt, at a cost of roughly USD 0.95 kWh, which is approximately four times the cost of current diesel generation in off-grid areas. Furthermore, the practical aspects of pumping algae from a separator to a dryer and handling and feeding algal solids into a combustor have not been standardized or automated for any commercial power generation scheme as of yet, so while this configuration appears quasi-attractive from a thermal efficiency, land footprint, and cost perspective, it would still require significant engineering input to be realized (Orosz and Forney 2008).

3.3 Thermochemical Processing

Thermochemical processing is the decomposition of organic materials from biomass for conversion at elevated temperatures and pressures into fuels. It comprises: pyrolysis, torrefaction, hydrothermal liquefaction, and gasification (Chen et al. 2015; Tan et al. 2014; Toledo-Cervantes and Morales 2014). Through these conversion technologies, solid, liquid, and gaseous biofuels are produced for heat and power generation. Pyrolysis is the combustion taking place at high temperatures (350–800 °C) in the absence of oxygen. It produces fuels with medium–low calorific power (Brennan and Owende 2010), such as charcoal, gas, and biocrude. Torrefaction is a mild pyrolysis at lower temperatures (200–300 °C) lasting minutes to hours, whose main product is a solid biofuel. Biocrude is also produced by hydrothermal liquefaction performed at 300–350 °C and pressures of 5–20 MPa to convert wet microalgal biomass into liquid fuel without using hot compressed or subcritical water (Chen et al. 2015). The refining of biocrude produces fuels and lubricants, and some of the byproducts form materials, such as plastics, detergents, solvents, elastomers, and fibers, such as nylon and polyesters, and asphalts (Chailleux et al. 2012). On the other hand, the main product of gasification is syngas (CO, CO_2, H_2), which is obtained when dry microalgae react with an oxidizer, such as air, oxygen, and water or steam, in a partial oxidation environment at a temperature ranging between 800 and 1000 °C, within a pressure range of 1–10 bar in an environment of insufficient oxidizer used for producing fuels and chemical intermediates. Comprehensive reviews of recent progresses and development of thermochemical processing are found in the latest literature (Toledo-Cervantes and Morales 2014; Chen et al. 2015; Chiaramonti et al. 2015).

3.3.1 Biochemical Processing

Biochemical conversion depends on the cell wall digestibility, which could be enhanced by physical, chemical, or biological pre-treatment of either whole or residual defatted biomass to reduce the processing time and increase the biomass. Pre-treatments are classified into physical, chemical, or biological. They include bead milling, ultrasound, alkaline, acidic or thermal hydrolysis, ionic liquid, pulsed electric field, microwave or enzymatic pre-treatment, among others (Eldalatony et al. 2016; Jankowska et al. 2017).

3.3.2 Anaerobic Digestion

Anaerobic digestion is the bacterial decomposition of organic biopolymers (i.e., carbohydrates, lipids, and proteins) into monomers in the absence of oxygen over a temperature range of about 30–65 °C. These monomers are easier to convert into a methane-rich gas via fermentation (typically 50–75% CH_4), and CO_2 is the second main component found in biogas (approximately 25–50%). Biogas can be upgraded up to >97% methane content and used as a substitute for natural gas (Toledo-Cervantes et al. 2017) to generate electricity.

The first mention of using microalgal biomass to produce biogas was long ago (Golueke et al. 1957), but the idea was taken to the modern times with the work of Sialve et al. (2009) and mainly due to the efforts to improve the economy and sustainability of biodiesel production from microalgae lipids (Harun et al. 2011) using the waste defatted biomass. Biogas production from anaerobic digestion of microalgae biomass is primarily affected by organic loads, temperatures, pH, and retention times in the reactor used. Besides, it was demonstrated that biogas potential is also strongly dependent on the microalgae species and biomass pre-treatment (Alzate et al. 2012; Jankowska et al. 2017; Harun et al. 2011; Mussgnug et al. 2010).

As was previously mentioned, biomethane from microalgae biomass can be used as gaseous fuel and to generate electricity, whereas the spent biomass can be used to make biofertilizers or a wide range of biofuels and chemicals in a thermochemical approach. Although microalgae biomass offers good potential for biogas production, industrial production has still not been fully implemented.

3.3.3 Fermentation

Bioethanol is usually obtained by alcoholic fermentation from carbohydrates, such as sugars, cellulose, or starch (Harun et al. 2014; Ho et al. 2013; Tan et al. 2014) or previously hydrolyzed lignocellulosic feedstocks. Microalgal bioethanol can be produced through two distinct processes: via dark fermentation or yeast fermentation.

The dark fermentation of microalgae consists of anaerobic bioethanol production by the microalgae themselves through the consumption of intracellular starch (Ueno et al. 1998). The yeast fermentation process of microalgal biomass is well known industrially, and to achieve higher yields, it is necessary to screen microalgal strains with high carbohydrate content or induce accumulation of intracellular starch. On the other hand, polysaccharides on the microalgal cell walls are not easily fermentable for bioethanol production by microorganisms. For fermentation, an acid pre-treatment has been proposed as the best option compared to other pre-treatment methods, namely in terms of cost-effectiveness and low energy consumption (Harun and Danquah 2011). During the bioethanol fermentation process, the pH is maintained in the range of 6–9, because a pH below 6 or above 9 could slow down bioethanol production. The fermentation process consumes less energy, and the process is much simpler in comparison with the biodiesel production system. In addition, the CO_2 produced as a by-product from the fermentation process can be recycled as carbon source for microalgae cultivation, thus reducing greenhouse gas emissions as well.

Hydrogen can be also produced by dark fermentation (DF) through the spore-forming bacteria, such as *Clostridium*. There are comprehensive reviews on hydrogen production from microalgae biomass (Buitrón et al. 2017; Sambusiti et al. 2015; Xia et al. 2015). Recent results show a clear potential of microalgae as feedstock for DF, achieving molar yields up to 3 mol H_2/mol sugar, which represents 75% of the maximum theoretical yield (Nayak et al. 2014). Such values are obtained only with other carbohydrate-rich substrates operated under thermophilic conditions or with a reduced hydrogen partial pressure. In DF, the highest yields are produced with the simplest carbohydrate molecules (Quemeneur et al. 2011); hence, carbohydrates must be released to be assimilated for hydrogen production when microalgae are used as substrate (Nguyen et al. 2010). Hydrolysis for cell wall disruption is a usual method to obtain fermentable sugars (Günerken et al. 2015).

Therefore, the major constraint to the use of microalgae for DF is related to the hydrolysate quality in terms of reducing sugar concentration and the pre-treatment efficiencies. Methane production is a frequent concern in DF systems because methanogenic microorganisms can be presented in the inoculum used. For instance, using wet untreated biomass, Kumar et al. (2016) produced methane rather than H_2 because of an inefficient inoculum heat pre-treatment.

As in other technologies for biofuel production using microalgae biomass, the suitable DF application depends on its insertion into an integrated scheme. The final by-product of DF is a mixture of volatile fatty acids and solvents, depending on the operational conditions and the microorganisms present.

3.3.4 Chemical transformation

Transesterification

Microalgae biodiesel is generally produced through the extraction and further transesterification of algal oil. Transesterification is the reaction of triglycerides (TAGs) with alcohol or methanol, in the presence of a catalyst that produces glycerol and fatty acid methyl esters (FAME or biodiesel) derived from TAGs. The complete biomass conversion depends on lipid profile, oil impurities, catalyst nature, temperature, and time. Transesterification can be catalyzed by acids, alkalis, or lipase enzymes (Chisti 2007). Recently, Lemões et al. (2016) have studied direct wet-transesterification using ethanol with yields similar to those obtained from extracted lipids. Furthermore, contributions to sustainability are claimed based on savings related to the unnecessary dewatering of the microalgae biomass, and the use of ethanol as renewable feedstock. Other innovation is transesterification in supercritical conditions, a catalyst-free chemical reaction that enables the full transformation of TAG (Ngamprasertsith and Sawangkeaw 2011), dramatically accelerated under supercritical conditions.

4 Biorefinery Strategies and Current Concepts

Fluctuations in fossil fuels prices, diminution of oil reserves, and COP21 agreements to reduce the GHG emissions encourage the biomass-biofuel industry and enhance the microalgae biofuel research (Pires 2017). Microalgae can play a dual role: They capture CO_2, and the resulting biomass can be used to produce a wide range of materials. It is important to mention that some chemicals derived from microalgae biomass cannot be synthesized from fossil fuels. Some of them are high-value products and can be used directly after separation or with slight structural adjustments. For these reasons, microalgae are of great interest for research and development of industrial processes to make viable their use in a biorefinery concept. As was mentioned previously, in the biorefinery concept, it is necessary to valorize most of the constituents of the microalgal biomass. Figure 3 shows options for valorization of the algae biomass that include its use as: (1) intact algae cells, (2) the disrupted whole cell content, or (3) fractionation of the biomass into different biochemical groups or specific compounds (Bastiaens et al. 2017). In the first alternative, the microalgae biomass is mostly commercialized as dry powder for feed, food, or aquaculture and the volume market is constantly increasing (IEA 2017; Vigani et al. 2015). Manufacturing commercial products derived from whole cells does not generate residual biomass, and extraction or fractioning of biomass is not necessary. On the contrary, the other two options allow the application of the biorefinery concept. The following sections present the main strategies to cultivate/process/valorize the microalgae biomass, and they are globally classified into four categories: (i) biofuels production only (low-value compounds), (ii) high-value-added products

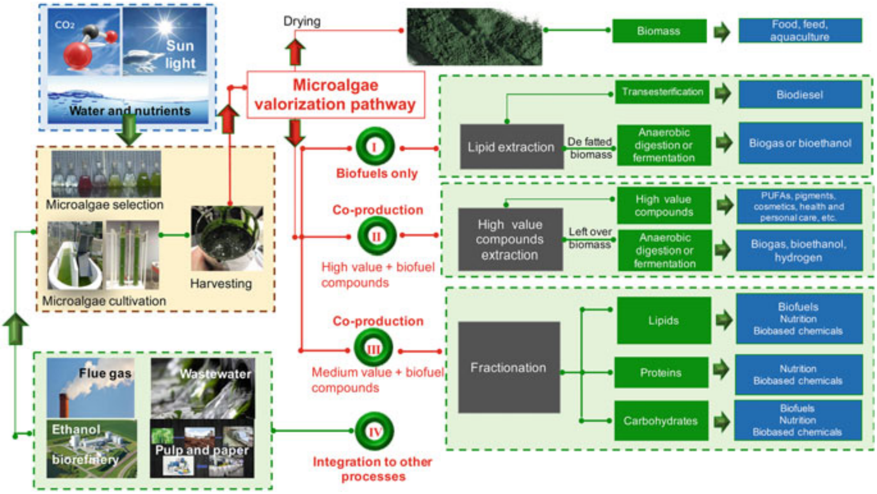

Fig. 3 Biorefinery strategies for microalgae biomass valorization

Table 5 Examples of biorefineries classified according the different strategies

Target compound	Substrate	System/conditions	Downstream processing	Production Scale	Market situation	Algae	References
Only biofuels							
Sugars (bioethanol) Residual biomass (biocrude)	BG-11 cultivation medium	Hybrid vertical flexible films, indoor and outdoor conditions	Vapor compression steam Stripper	Pilot scale	Marketable products distributed by Algenol Company	Cyanobacteria	Sterner (2013)
Lipids (biodiesel) Residual biomass (biomethane)	Post-transesterified microalgae residues (*Chlorella* sp.)	Anaerobic digester 2 L, continuous stirring at 300 rpm, semi-continuous fed	Anaerobic digestion	Laboratory	–	Digester bacteria	Ehimen et al. (2011)
Lipids (biodiesel)	Bold's Basal Medium	Raceway pond, 2000 L, greenhouse conditions, semi-continuous mode	–	Pilot scale	–	*Scenedesmus* sp. SCX2	Fernández-Linares et al. (2017)
Lipids (biodiesel) Residual biomass (methane)	Liophilized biomass	–	SCF extraction with CO_2, anaerobic digestion	Laboratory	–	*Scenedesmus almeriensis*	Hernández et al. (2014)
Lipids (biodiesel) Carbohydrates (bioethanol)	Codafol 14.6.5 plant fertilizer	Plastic sleeves, 300 L, outdoor conditions	Cell disruption by steam explosion and solvent extraction with hexane	Pilot plant	–	*Nannochloropsis gaditana*	Nurra et al. (2014)
Lipids (biodiesel)	Wastewater and gas emissions from refinery oil industry	Bubble column photobioreactor, 2 L	Lipid extraction by Bling and Dryer method	Laboratory	–	*Aphanothece microscopica Nägeli*	Jacob-Lopes and Franco (2013)

(continued)

Table 5 (continued)

Target compound	Substrate	System/conditions	Downstream processing	Production Scale	Market situation	Algae	References
Hydrogen residual biomass Biogas	TAP medium and ProF medium	–	Drying and anaerobic fermentation	Laboratory	–	*Chlamydomonas reinhardtii* and *Scenedesmus obliquus*	Mussgnug et al. (2010)
Biofuels and high-value compounds							
Carotenoid (pigments) Lipids (biodiesel)	Mineral medium culture	Bubble column 5L light intensity 25.7 µmol/m^2/s and temperature of 25 °C	Freeze-drying and milling and acetone, and Sohxlet extraction with hexane	Laboratory	–	*Chlorella protothecoides*	Campenni et al. (2013)
PHB (bioplastic) Lipids (biodiesel) Residual biomass (biogas)	Mineral medium culture	Tubular reactor	Solvent extraction with hexane, Glycerol purification	Model conceptual	–	*Haematococcus pluvialis*	García-Prieto et al. (2014)
Carotenoids (pigments) Lipids (biodiesel) Residual biomass (hydrogen)	GPM medium	Polyethylene bag, 10 L, light intensity 25.7 µmol/m^2/s and temperature of 25 °C	SCF extraction with CO_2/ethanol, dark fermentation	Laboratory	–	*Nannochloropsis* sp.	Nobre et al. (2013)
β-carotene, phytosterols fatty acids and biochar	Walne's medium	Vertical tubular photobioreactor, 400L, outdoor conditions, semi-continuous mode	Pyrolysis	Pilot scale	STAR facility center	*Dunaliella tertiolecta*	Francavilla et al. (2015)

(continued)

Table 5 (continued)

Target compound	Substrate	System/conditions	Downstream processing	Production Scale	Market situation	Algae	References
Biofuels and other medium products							
Lipids (biodiesel) Proteins (food) Reduced sugars (bioethanol)	BG-11 mineral medium	Raceway 300 m^3, outdoor conditions	Pre-treatment, cell disruption Lysis buffer solution for protein and H_2SO_4 and autoclaving for reduce sugars	Laboratory	–	*Scenedesmus obliquus*	Ansari et al. (2015)
Lipids (biodiesel) Protein (food or feed) Carbohydrates (polymers)	Domestic wastewater	Reactor 25L, with 16:8 h, Light-dark (80 $\mu mol/m^2/s$) cycle and temperature of 25 °C	Sequential biomass extraction (protein, lipids and carbohydrates)	Laboratory	–	*Scenedesmus obliquus*	Ansari et al. (2017)
Sugars (bioethanol) Lipids (biodiesel) Proteins (feed)	Mineral medium culture	Flat panel photobioreactor, 650 L, outdoor conditions	Acid pre-treatment, solid liquid separation, fermentation, solvent extraction	Pilot scale	High-value coproduct opportunities possess potential to significantly reduce the high cost	*Scenedesmus acutus*	Dong et al. (2016)

(continued)

Table 5 (continued)

Target compound	Substrate	System/conditions	Downstream processing	Production Scale	Market situation	Algae	References
Fucoxanthin carbohydrates and proteins with antioxidant activity	Mineral medium culture	Vertical photobioreactor, 400 L, outdoor conditions	(1) SCF extraction with CO_2, (2) SCF extraction with CO_2/ethanol, (3) pressurized liquid extraction (PLE) with ethanol, (4) PLE with pure water	Pilot scale	–	*Isochrysis galbana*	Gilbert-López et al. (2015)
Proteins	–	Horizontal tubular photobioreactor, 2000 L, outdoor conditions	Cell disruption by enzymatic treatment and ultrafiltration/ diafiltration	Laboratory	–	*Nannochloropsis gaditana*	Safi et al. (2017)
Biorefinery integration with other process							
Residual biomass organic acids and biogas Lipids (biodiesel) Sugars (bioethanol)	Mineral medium culture	Mathematical model developed in MATLAB Open pond, environmental conditions	Pre-treatment, cell disruption, supercritical fluid extraction (SCF) with CO_2 and ethanol as a co-solvent	Laboratory	Still not economic profitable	*Chlorella vulgaris*	Albarelli et al. (2017)
Lipids (biodiesel) Fatty acids (food) Proteins (feed)	Wastewater	Photobioreactors and open ponds, the LCA represents 100-ha facility	Membrane filtration, hexane extraction,	Demonstration facility	Cellna’s Kona Demonstration Facility	*Nannochloropsis oceanica*	Barr and Landis (2017)

(continued)

Table 5 (continued)

Target compound	Substrate	System/conditions	Downstream processing	Production Scale	Market situation	Algae	References
Lipids (biodiesel) Residual biomass	Mixed municipal and industrial wastewater	Laboratory tubes	Drying, lipids extraction through Folch method	Laboratory-scale	–	*Scenedesmus* sp.	Gentili (2014)
Residual biomass (agricultural or food industry) Hydrogen	Residual biomass for dark fermentation and wastewater for algae culture	PBR and anaerobic digester for dark fermentation PBR (photo-fermentation)	Anaerobic digestion (dark fermentation)	Laboratory-scale	–	*Chlamydomonas reinhardtii* *Scenedesmus obliquus* *Chlorococcum littorale* *Platymonas subcordiformis* *Chlorella fusca*	Kapdan and Kargi (2006)
Organic acids	Microalgal biomass used as substrate	Incubator chamber	Alkaline pre-treatment, enzymatic saccharification and anaerobic digestion with Clostridium	Laboratory	–	*Chlorella* sp. and *Tetraselmis suecica*	Kassim and Meng (2017)
High-value microalgae products, fertilizer, and biogas	Secondary streams from pulp and paper industry	Conceptual configuration, liquid effluent from the mechanical dewatering of digested residues and flue gases from biomass boiler at the mill		Conceptual biorefinery CO_2 Wastewater	–	*Nannochloropsis*	Kouhia et al. (2015)

(continued)

Table 5 (continued)

Target compound	Substrate	System/conditions	Downstream processing	Production Scale	Market situation	Algae	References
Joint production of sugar, ethanol, and electricity from sugar cane biorefinery and biodiesel and glycerol from microalgae	Cultivation using CO_2-rich streams derived from fermentation and cogeneration system	Conceptual configuration and simulation of scenarios including economic evaluation		Conceptual biorefinery under the knowledge-based approach CO_2	–	*Chlorella* sp.	Moncada et al. (2014)
Lipids (biodiesel) Protein (food) Pigments (food) Carbohydrates (bioethanol)	Wastewater from fish processing industry	Bubble column photobioreactor, 5 L	Coagulation–flocculation–sedimentation and membrane microfiltration	Laboratory	–	*Aphanothece microscopica Nägeli*	Queiroz et al. (2013)
Lipids (biodiesel) Residual biomass (biosyngas and methanol)	CO_2-flue gas from a coal energy plant	–	Oil extraction, gasification of remaining biomass to obtain syngas conversion of biosyngas to methanol	Conceptual model	–	*Chlorella pyrenoidosa*	Wiesberg et al. (2017)

and biofuels, (iii) medium-value-added products (bulk compound) plus biofuels, and finally, (iv) coupled to other processes in the context of circular economy. Illustrative works about these categories are shown in Table 5 and Fig. 3.

4.1 Strategy I: Biofuel Production Only (Low-Value Compound)

Biofuels are required at low price and in large volume; however, among all products obtained from microalgae, biofuels have the lowest cost and their price is always compared with fossil alternatives; moreover, the energy balance of the production process must be positive. As was shown in Sect. 2 and Table 5, most of the biofuels can be conceptually obtained from microalgae, but biodiesel is one of the most interesting alternatives as liquid fuel for transportation (Amaro et al. 2011; Mallick et al. 2016; Mondal et al. 2017). Nonetheless, it has been demonstrated that currently microalgae biodiesel is not cost competitive when compared with fossil diesel (Chisti 2007). Therefore, the idea of using the residual biomass to obtain more energy was introduced looking for a better revenue, by minimizing wastes to complete biomass use in the biorefinery concept (Collet et al. 2011; Maurya et al. 2016). In the strategy of biofuel only, there are three scenarios to obtain more energy: (i) the wastes can be burned to generate heat and electricity, (ii) they can be converted to biogas using anaerobic digestion or to ethanol using fermentation from carbohydrate, and (iii) they can be thermochemically processed to obtain other biofuels (Brownbridge et al. 2014). But in the structure of the biorefinery with bioprocessing only, selection will depend on the stoichiometric composition of the cell; lipid content in the cell can vary from 15 to 80% depending on the strain, leaving over a huge amount of wastes. Residual deoiled biomass composition is rich in carbohydrates or proteins, and its composition defines the potential application. Residual biomass with high C/N ratio is beneficial for the production of biomethane, bioethanol, and biohydrogen, while a low C/N ratio means high protein content, which may be beneficial for its use as fertilizer, fermentation medium for microorganisms, or feed supplement for animals and fish (Maurya et al. 2016). Biogas production from residual deoiled biomass was suggested to produce heat or electricity and contribute to the energy balance (Collet et al. 2011; Ward 2015). Laurens et al. (2017b) established that fractionation approach to microalgal biomass can create three different potential fuel streams, which allow a 35% reduction in the overall minimum fuel selling price as compared to the biodiesel-only strategy. As can be seen from Table 5, the general strategy is focused on exploiting lipids for biodiesel production and there is a preference for biogas production first from residual biomass and then from bioethanol. One strategy attempts to diversify its production of biofuels with hydrogen. In this approach, ethanol is obtained from fermentation of the released carbohydrates, biodiesel, or jet fuel from the lipid fraction through hydrotreating and isomerization and finally mixed alcohols (isobutanol, isopentanol, and others) from the protein fraction. However, it becomes clear that the use of residual biomass alone to produce energy is not as favorable as it looks at first glance.

4.2 Strategy II: Coproduction of High-Value-Added Products and Biofuels

Microalgae are important producers of many high-value nutraceutical compounds, such as polyunsaturated fatty acids or astaxanthin that can justify the high cost of microalgae cultivation and processing technologies (Liang et al. 2015; Shah et al. 2016). Under this scenario, the fixed CO_2 is valorized and biofuels are produced after extraction of high-value products. Some examples include astaxanthin produced by *Haematococcus pluvialis*, this high-value-added molecule is already commercialized (Lorenz and Cysewski 2000), and its market price is 7000 USD/kg (Hariskos and Posten 2014; Shah et al. 2016). This microalga is an excellent candidate for this strategy because astaxanthin accounts for approximately 5% of the total cell dry weight, representing only a small fraction of it. Astaxanthin is produced under nitrogen limitation and simultaneously with triglycerides that constitute up to 60% of dry weight (Solovchenko 2015) and can be utilized for biodiesel. In this way, after astaxanthin extraction, biodiesel can be produced from lipids and biogas from residual biomass (Shah et al. 2016). Another organism suitable for the biorefinery of high-value-added compound is *Nannochloropsis* (Chua and Schenk 2017) due to its rapid growth, high oil productivities, and omega-3 fatty acid content, specifically the EPA whose market price is up to USD 100 per liter. Furthermore, in the biorefinery configuration, the high edible protein content makes this alga feasible for food or feed. Other high-value molecules in microalgae biomass are: vitamins, pigments, etc., and other examples of studies exploring this strategy are shown in Table 5. As can be seen, pigment as high-value compound coupled to production of biodiesel from lipid fraction or other biofuels from the leftover biomass is the most commonly studied scenario.

One of the major challenges of this strategy is that despite the fact that the projected demand for high-value products from microalgae is increasing, these products are still produced in relatively small amounts; therefore, the biofuel supply cannot be guaranteed.

4.3 Strategy III: Coproduction of Medium-Value-Added Compounds and Biofuels

The approach of integration of medium-value products (carbohydrates, lipids, and proteins) into biofuels production was proposed by Wijffels et al. (2010).

They emphasized that a process for biodiesel from microalgae lipid production only is unlikely to be economically viable and that all biomass bulk components should be valorized in order to develop a feasible and sustainable process. This idea promotes diversification of market sectors, introducing microalgae products not only in the energy sector. According to Hariskos and Posten (2014), bulk chemicals constitute a market volume of >10,000 tons/year with prices from only a few USD/kg up to 100 USD/kg and represent 11% of crude oil destined to petrochemical synthesis. As was seen before, the main components of microalgal biomass depend on the strain and common contents are: lipids (30–50%), proteins (50–70%), carbohydrates (50%), and pigments (Chew et al. 2017). These biochemicals involve the use of protein for feed or food; carbohydrates for bioactive materials, cosmetics, nutritional, and pharmaceutical applications; and lipids, which depending on the length chain, have application as surfactants, cosmetics, solvents, lubricants, or biopolymers (Hariskos and Posten 2014; IEA 2017). Most of the studies focused mainly on lipids for biodiesel and proteins for food or feed. But others include bioethanol production (Table 5).

Under the coproduction strategies (II and III), the selection of mild and selective separation techniques is important to keep the properties of the most biomass components. Therefore, an adequate progression of harvesting followed by cell disruption (Lee et al. 2012) and a further suitable mild and selective extraction and purification sequence of metabolites of interest must be chosen.

Regarding the definition of the best order of extraction of metabolites, it depends on the strain and properties of the products to be recovered. Ansari et al. (2017) showed for *Scenedesmus obliquus* that the sequence of extraction: proteins–lipids–carbohydrates was the most convenient. However a different strategy was proposed by Dong et al. (2016), who suggested that a combined algal processing (CAP) is much better than parallel algal processing (PAP). Instead of extracting lipids from algal biomass prior to alcoholic fermentation as in PAP, lipids are extracted from the anaerobic digestion cake. CAP turns out to be highly efficient for sugar conversion, and lipid loss is negligible. CAP reduces the biofuel cost of microalgae by 9%. However, it is important to mention that this study did not evaluate the cost of bioproducts in a complete biorefinery scheme, using the whole biomass. Table 5 shows different studies using this strategy.

Under this strategy, the high efficiency of fractionation in a sequential process is one of the principal bottlenecks and represents a challenge to overcome. The main goal of this approach is to maximize biomass production and valorization in order to prioritize the use of biomass to obtain products of value by giving more importance to the production of materials, rather than its use for energy (Keegan et al. 2013).

4.4 Strategy IV: Integration of Microalgae into Other Processes and Circular Economy

In recent years, the importance of the new concepts for biorefineries and in particular for microalgae's has been highlighted. Biorefinery obeys the principles of a circular economy in the sense that all waste streams are valued (Mohan et al. 2016a) promoting the use/transformation of secondary or residual streams into value-added products (Yuan et al. 2015). It includes the use of: (i) wastewater as nutrient source (Barr and Landis 2017; Delrue et al. 2016; Gouveia, et al. 2016; Olguin 2012; Queiroz et al. 2013; Zhu 2015), (ii) digestate from a wastewater treatment of the pulp and paper industry to internally recycle the nutrients for microalgae cultivation; this is an example that leads to a notably lower cost of microalgae biomass production (Kouhia et al. 2015) or (iii) gaseous waste streams with high CO_2 content (Moncada et al. 2014; Wiesberg et al. 2017).

On the other hand, in all the above-mentioned studies, the potential of microalgae for producing different forms of bioenergy and chemicals has been presented as separated concepts that are not integrated into the first and second biorefineries in multiproduct portfolios. Recently, it has been recognized that the biorefinery concept plays an important role in the future development of a bio-based economy and integration of the first-, second-, and third-generation biorefineries has been recently proposed to develop a complete bioindustry (Moncada et al. 2014). That work analyzed the integration of microalgae into a second-generation sugarcane biorefinery, including the joint production of sugar, ethanol, and electricity, by introducing the cultivation of microalga *Chlorella* to use CO_2-rich streams derived from fermentation and cogeneration systems and subsequently produce biodiesel, glycerol, sugar, fuel ethanol, heat, and power. Table 5 shows the concept of incorporation of microalgae into other biorefinery or production schemes. These scenarios are as diverse as the existing types of biomass; some works include microalgae in pulp and paper industry or sugar cane biorefineries for treatment and valorization of effluents, using combustion gas or wastewater as presented in Table 5.

It is important to note that under this scenario, the possibility of biorefinery schemes is infinite. Despite the complexity it involves, the biorefinery needs to promote a circular economy to achieve viability (Mohan et al. 2016a, b). In the proposals, the concept of biorefining for one single product was abandoned.

4.5 Design Strategy and Current Status

In order to conceptualize a biorefinery, the sequence of decisions includes (Toledo-Cervantes and Morales 2014): (1) microalgae rich in target products. (2) cultivation conditions and operation strategies, (3) conversion processes of whole microalgae/defatted microalgal biomass to biofuels, (4) biomass harvesting, post-harvesting technology, (5) methods and sequence of extraction of coproducts/processing the whole biomass/the pre-extracted product to final products, (6) process integration of streams and recycling of materials, reducing wastes, and finally (7) a life cycle analysis. In the case of high-value products, Eppink et al. (2017) suggested specific recommendations about harvesting, cell disruption, and extraction methods. They indicated the need to know the composition and strength of the cell wall, and localization of the target compound in the cytoplasm, as well as to select moderated harvesting methods and mild cell disruption, removing first the cell wall and after, the organelle disruption. The next step is the selective separation of hydrophobic (lipids, pigments) and hydrophilic (proteins, carbohydrates) fractions while keeping full functionality and finally, fractioning the hydrophilic and hydrophobic component mixtures to recover target compounds for different market applications. The final step for the implementation of a biorefinery process is to connect different stages into the complete chain process. All efforts in designing multiproduct scenarios are looking to increase the economic feasibility of global production of biofuels, but, as can be seen, a biorefinery configuration involves many decisions to be made, including alternatives for cultivation, water consumption and nutrients supply, culture condition to trigger accumulation target products, and a complicated downstream processing to maximize the product recovery-exploitation. Therefore, in order to define the adequate processing pathway of the microalgae biomass, different scenarios have to be analyzed using optimization techniques, including techno-economic aspects and sustainability issues.

5 Economic and Sustainability Aspects

Detailed information and fundamentals about economic aspects and life cycle assessment (LCA) of microalgae biofuels will be presented in Chaps. 6 and 7 of this book, and this section is specific for the biorefinery strategy.

Most of the current research about microalgae biofuels is based on the potential of biodiesel from microalgae lipids (Chisti 2007); this optimistic study was performed in an extraordinary situation with extremely high oil prices, favoring the biofuel development. At that time, the initial challenge to decrease its production cost to 0.48 USD/L was established. Afterward, Wijffels et al. (2010) established that this biodiesel price was achievable, when considering a microalgae biorefinery, based on the valorization of different biochemical fractions and high-value-added products. Up to date, several studies have been published on the economic analysis of microalgae-based processes (Acién et al. 2017; Davis et al. 2016; Douskova et al. 2009; Hoffman et al. 2017; Norsker et al. 2011; Slade and Bauen 2013; Tredici et al. 2015). Recently, reviews about techno-economic evaluations of microalgae biofuels, from a biorefinery point of view, were performed by analyzing a great variety of scenarios (de Boer and Bahri 2015; Laurens et al. 2017b). Those works report prices ranging from 0.88 USD/L up to 24.60 USD/L, concluding, on the basis of available techno-economic studies and current technologies, that microalgal biofuel production is 4–5 times more expensive than current fossil fuels; and actions to reduce that cost involve: (1) productivities of at least 30 g/m^2/day and minimum lipid content of 30%, (2) lowering the capital cost, discarding the use of photobioreactors and centrifuges, reducing costs of dewatering, and finally identifying opportunities for lower-cost carbon and nutrient sources.

In addition to economic evaluation, biofuels from microalgae must also meet favorable life cycle goals on energy return, and carbon and water footprint to provide quantitative improvements to current fuels.

However, there is no common conclusion on sustainability of microalgae biofuels (Gnansounou and Raman 2017; Quinn and Davis 2015). The significant variance in the studies could be due to diverse choices regarding technical (microalgae species, production units, downstream processing, and technology for energy production, coproducts) and methodological alternatives (functional units, boundaries, coproduct allocation methods) (Collet et al. 2015; Thomassen et al. 2017). But there is a general agreement that producing only biodiesel from algae is not favorable and, in order to reduce the overall cost, the following have been suggested: (i) process integration (CO_2 capture, wastewater treatment, and biofuel production); (ii) optimization of photobioreactor design and conditions to improve biofuel yield; and (iii) extraction of valuable products from algal biomass (biorefinery concept). Therefore, a multiproduct strategy in a biorefinery is indicated as the future trend. Nevertheless, the absence of facilities for microalgae biofuels production at industrial scale with accurate/reliable information entails theoretical assumptions or extrapolation of laboratory information to make predictions, whereas the design problem is mathematically formulated to describe the production systems and its performance. Recent theoretical studies about economic aspects or LCA in a multiprocessing-downstream processing-multiproduct strategy have been published (Gutiérrez-Arriaga et al. 2014; Martinez-Hernandez et al. 2013; Menetrez 2012; Posada et al. 2016). Also, multiobjective optimization approaches to trade off different criteria simultaneously have been performed (Andiappan et al. 2014; Brunet et al. 2015; Rizwan et al. 2015; Santibañez-Aguilar et al. 2014) by applying mixed integer nonlinear programming (MINLP) models or Monte Carlo simulations to maximize incomes or production yields, determine economic viability, and minimize the environmental impact to find the optimal processing pathway for the production of biodiesel from microalgal biomass and treating wastes. Although computational tools are developed, no scenario has reached 0.48 USD/L necessary to compete with the fossil alternative.

In general, most LCA studies concluded that bioenergy from algae has lower greenhouse gas (GHG) emissions than fossil fuels and that energetically viable process must use raceway ponds, process wet biomass (avoid drying), minimize energy required for cell disruption, and minimize solvent use (de Boer and Bahri 2015).

6 Remarks and Conclusion

The lineal system of our current economy (extraction, manufacture, use, and disposal) has reached its limits, entailing depletion of a number of natural resources and fossil fuels (Mohan et al. 2016a). In a bio-based economy, biorefinery strategy is a key factor to close the loop in a circular economy with a restorative and regenerative production model that values waste and minimizes negative environmental impacts through a transition to renewable energy sources. Microalgae biomass is one of the best alternatives for a biorefinery due to the diverse products that can be obtained. However, current applications of microalgae biomass are mainly for food and feed, and biofuels are not produced at industrial scale. At present, the high production cost of biomass and its subsequent fractionation make it economically nonviable, particularly when the production focuses on a single product, such as fuel. Advances in genetic modification of strains, production systems, and downstream processing, besides valorization and acceptance of a broad range of products, could contribute to assess sustainability and profitability.

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