Chapter 5

Microalgal Biorefineries

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1. Introduction

The world has been confronted with a food and energy crisis due to accelerated global population growth and the depletion of finite fossil fuel resources. The increase in nutritional problems along with rising fuel demands and environmental problems have necessitated the search for nutritional supplements and sustainable sources of energy. Currently, fossil fuel resources are not regarded as sustainable and their continued consumption is raising serious ecological, economic and environmental questions. However, while we move towards alternative sources of energy, there remains a need to replace fossil fuels with high energy density fuels. A highly contentious issue of great concern is the argument that emissions of carbon dioxide (CO_2) from fossil fuel use, especially from coal combustion, are responsible for global climate change. As a result of studies during the past five decades, and most notably from the last 20 years, emissions of CO_2 have become an important issue with respect to global climate change because atmospheric CO_2 concentrations increased significantly in the last century and have continued to rise at an increasing rate [1].

The United Nations Kyoto Protocol of 1997 established regulations designed to control emissions of air pollutants with the objective of reducing greenhouse gases to the level of emissions in 1990, and more than 170 countries have ratified the protocol [2].

Various CO_2 sequestration techniques have been developed and the various technologies for CO_2 capture and storage need to be evaluated from the point of view of obtaining carbon credits, aimed at stabilizing emissions of the pollutant [2]. Of these techniques, CO_2 capture by photosynthetic organisms such as microalgae shows good potential in view of the economic advantages it presents, rate of CO_2 capture, and the speed with which the technology can be introduced to the industrial community.

Microalgae have great potential in generating energy from biotechnological processes using renewable sources and without compromising food security and agriculture. Microalgae have been of major interest in biofuel production as well as in the feed, chemical and pharmaceutical sectors [3]. Depending on the species and growth conditions, microalgae can be selected to produce a wide variety and abundance of lipids, proteins, carbohydrates, and feedstocks important for biofuel and production of nutraceuticals [3].

The rapid growth rate coupled with high productivity from a small area means that the production of microalgal biomass has a promising future [4].

Several investigations into the use of microalgae to obtain bioproducts have been successfully conducted. Upstream processing (USP) and downstream processing (DSP) are stages found in the processes of microalgae biorefineries (Fig. 1). USP involve four important areas: (i) microalgae strain, (ii) CO_2 supply, (iii) nutrient source <nitrogen/phosphorus> and (iv) source of illumination [5].

Photobioreactors used for the culture of microalgae are of two basic designs — open or closed systems. Amongst the different types of open system design, the most popular is the raceway pond, while popular closed systems include flat-plate, vertical tubular, horizontal tubular and hybrid type photobioreactors. Growth of microalgae in photobioreactors occurs due to the use of CO_2 rich gas as a means of mixing, as well as being a source of carbon. Generally, in this type of reactor, the agitation, mass transfer, efficient provision of light, removal of photosynthetically generated oxygen, understanding of hydrodynamic aspects, and scalable photobioreactor technology are aspects that should be taken into account to achieve good yields [6,7].

Conventional DSP includes all unit processes that follow the process taking place within the photobioreactor. They involve biomass harvesting and biorefinery techniques which facilitate the integration of the biomass conversion processes and equipment for the production of several fractions of interest through the use of mild separation technology. Biorefining involves assessment and use of different technologies to obtain different types of bioproducts from biomass, which can be marketed and used to solve specific problems in many different areas. Finally, there must be the safe and inexpensive disposal of all waste products generated during the process. Therefore, a portion of the residual biomass can go to an anaerobic digester to generate biogas, and the rest can be used as nutrients to feed the photobioreactor again.

As such, the aim of this chapter is to present an overview of the potential uses of the technology in the transformation of carbon dioxide into biomolecules, and to describe the processes involved in the biological conversion of CO_2 in photobioreactors as well as biorefinery techniques suitable for the treatment of microalgal biomass and the production of biomolecules.

2. Carbon dioxide emissions and mitigation

Climate change occurs mainly due to increased levels of CO_2 in the atmosphere. During the twentieth century an increase in CO_2 concentration of 30% was observed. This rate of increase

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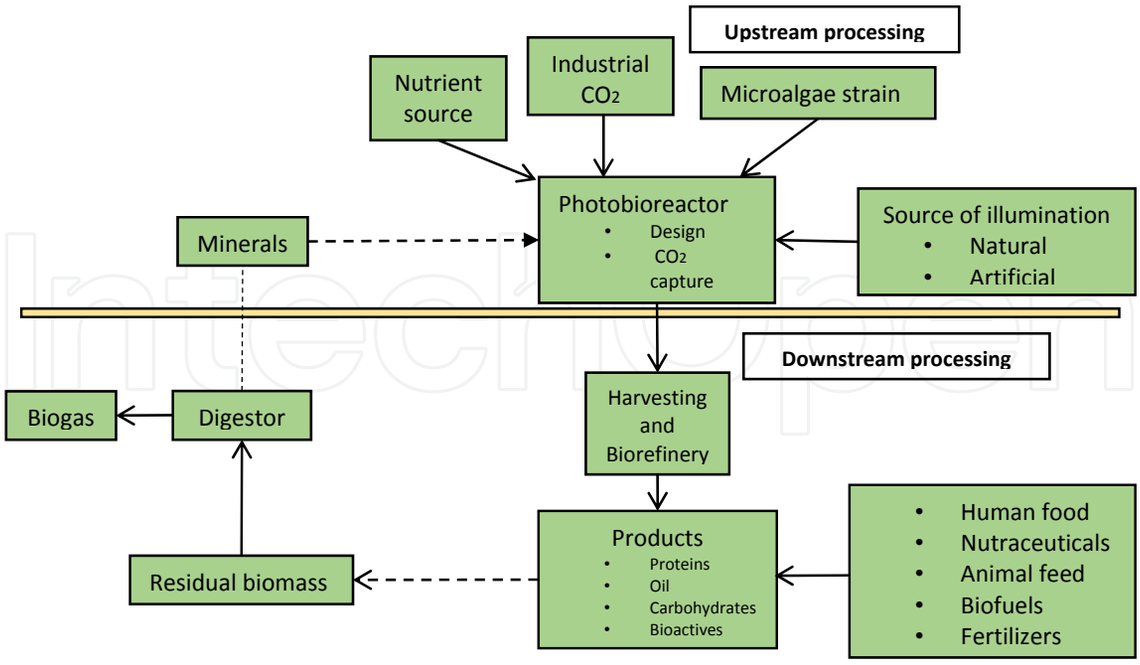

Figure 1. Outline of the formation process of microalgal biomass and bioproducts.

will lead to an increase in CO_2 levels of 49% by the end of this century [8]. CO_2 emissions from fossil fuel combustion saw an increase of 41% between 1990 and 2008 [9]. Three potential sources of CO_2 can be found: stationary, mobile and natural. CO_2 is the primary greenhouse gas emitted through human activities. Stationary sources contribute the highest percentage of CO_2 emissions of these, and are anthropogenic in origin, such as from industrial or domestic processes. The industrial processes contributing to increasing atmospheric CO_2 concentrations consist of hydrogen and ammonia production plants, power stations, cement companies, ethanol companies, and chemical factories. Flue gases from power plants are responsible for more than 8% of the world's total CO_2 emissions. Mobile sources are those from transport, while natural sources include volcanoes and elements of human or animal decomposition [10].

A number of research and development efforts have been directed at reducing CO_2 emissions. Many of these studies use different microalgae strains or new photobioreactors with geometric configurations that may be a fundamental step forward for the consolidation of this technology [11-14].

Biological methods for CO_2 mitigation can be carried out by photosynthetic microorganisms such as microalgae and plants, the latter with an estimate for CO_2 capture of only 3–6% of fossil fuel emissions [15]. Biomitigation using microalgae as a method of decreasing CO_2 emissions by fixing CO_2 through photosynthesis is considered one of the most effective. Generally, microalgae have higher growth rates, a higher CO_2 fixation efficiency and a larger production of biomass enabling the subsequent development of quantities of bioproducts with high added value [16].

However, much the flue gas of industrial origin, in addition to contributing to CO_2 emissions, produces other compounds, including oxygen (O_2), water vapour, carbon monoxide (CO),

nitrogen oxides (NOx), sulfur oxides (SOx), hydrochloric acid, heavy metals, and particulate matter [17]. These compounds have a toxic and/or inhibitory effect on microalgal growth. Tolerance of microalgae to elements of flue gas is dependent on the strain. NOx present in flue gas can be taken up and could become an alternative nitrogen source for the growth of microalgae growth. The main impact is due to SOx which reacts with water to form sulfurous acids. This can be prevented by buffering or active pH control [18].

Besides high amounts of NOx and SOx, the high temperature influences the growth of microalgae. Industrial plants discharge flue gas at a temperature between 70–120 °C [19]. Therefore, to complete the CO_2 capture process, it is necessary either to install a post-cooling system, or to use thermophilic species [18]. Additionally, oxidant compounds found in flue gas can cause damage to proteins and pigments, and compromise the integrity of cell membranes [20].

Besides the use of CO_2 from industrial flue gas, other alternative sources are known such as ethanol production facilities, winegrowing, ammonia and hydrogen production or gas-processing plants. The capture of CO_2 from the fermentation process is relatively simple and cheap due to the higher state of purity in which it is present [21].

Algae companies	Country	Description	Ref
AFS BioOil Co.	San Francisco, USA	Founded in 2010. This company uses algae that are fed by nutrients recovered from wastewater treatment plants, electricity generation, and sunlight. Biodiesel, the main product produced in these biorefineries is cost competitive with petroleum products.	[22]
AFS Biofarm™	San Francisco, USA	Uses CO_2 sequestered from industrial facilities and power plants for conversion into renewable fuels and other valuable products such as food additives.	[23]
Seambiotic Ltd.	Tel Aviv, Israel	Founded in 2003. The company aims to develop microalgae biomass for the production of food additives and biofuel using flue gas from coal burning power stations.	[24]
Aeon Biogroup	Chile	This company develops biomass production methods with CO_2 capture from winegrowing for production of oil, nutraceuticals, food additives and biochemical compounds.	[25]
IGV Biotech	Nuthetal, Germany	Founded in 1960. IGV Biotech develops microalgae biotechnology processes for the production of several products such as food, pharmaceuticals and chemicals. This company uses advanced technology for the cultivation of photosynthetic microorganisms and CO_2 capture.	[26]
Algenol Biofuels	Florida, USA	Founded in 2006. The company uses CO_2 and seawater as a culture medium for bioethanol. Nitrogen fixing technology is used to reduce production costs of fertilizers by cyanobacteria.	[27]

Table 1. Global companies with CO_2 sequestering technology for algae culture.

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Table 1 shows some global companies which employ CO_2 sequestering technologies for the production of biofuel and/or bioproducts from algal cultures. Furthermore, many companies and research centers worldwide are investigating the upstream and/or downstream process.

3. Microalgae strains and photosynthetic metabolism

Microalgae are fast growing photosynthetic microorganisms that produce valuable compounds, are easy to harvest, exhibit a unicellular or simple multicellular structure, and a large surface-to-volume body ratio. Eukaryotic microalgae such as green algae (*Chlorophyta*) and diatoms (*Bacillariophyta*) as well as prokaryotes like cyanobacteria species (*Cyanophyceae*) use oxygenic photosynthesis to fix CO_2 like macroalgae and plants [18].

CO_2 fixation, biomass production and bioproduct diversity vary with microalgal species, although the data may not be strictly comparable as the microalgae may have different biological behavior or may have been cultured in different conditions. The general chemical composition of different microalgal species varies, with some species having greater potential for the production of certain bioproducts [28]. Microalgae have a varied biochemical profile (Table 2). The high protein content of microalgae species is notable. These proteins, mainly amino acids, provide nutritional elements that can meet food requirements in humans and animals.

Consequently, a successful and economically viable microalgae industry producing bioproducts mainly depends on the selection of appropriate microalgae strains.

Microalgae comprise a diversity of species characterized by a variety of phenotypes dependent on their pigments and cell structure. Chlorophyll-*a* and phycobiliproteins may be present and are involved in harvesting light energy for photosynthesis. They are, therefore, a good choice for the generation of biomass. In addition to photosynthesis, some species show an ability to adapt to different environments and metabolisms such as respiration and nitrogen fixation, chromatic adaptation and the ability to form symbiotic associations with yeast, fungi, bacteria and plants [31]. The part of the photosynthetic process in which CO_2 is converted into carbohydrates is catalyzed by the carboxylase activity of the enzyme ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO), this step is called the Calvin cycle [32]. The Calvin cycle is the metabolic mechanism for CO_2 fixation in microalgae and the process comprises three phases: carboxylation, reduction and regeneration. The photosystem II (PSII) complex is the starting point of photosynthesis, where via the electron transport chain, an electron is transferred to cytochrome b6f and PSI. A proton-motive force is created due to the pumping of electrons in opposite directions, creating a difference in charge across the membrane. This is used for ATP synthesis and the formation of ferredoxin and NADPH. The electron is donated by water and oxygen is formed as a waste product [33,34]. To generate one molecule of carbohydrate (CH_2O), O_2 and H_2, at least eight (8) photons are needed. The mean energy content for photosynthetically active radiation (PAR) photons is close to 220 kJ/mol and the total potential light energy captured by photosynthesis is 1744 kJ/mol of CH_2O. The theoretical

Microalgae species	Protein	lipid	Carbohydrate	Nucleic acid	Ref.
Anabaena cylindrical	43–56	4–7	25–30	–	[28]
Aphanothece microscopica Nägeli	41–49	8–9	13–18	3–4	[29]
Arthrospira maxima	60–71	6–7	13–16	–	[28]
Chlamydomonas rheinhardii	48	21	17	–	[28]
Chlorella pyrenoidosa	57	2	26	4–5	[28]
Chlorella vulgaris	51–58	14–22	12–17	4–5	[28]
Dunaliella bioculata	49	8	4	–	[30]
Dunaliella salina	57	6	32	–	[30]
Euglena gracilis	39–61	14–20	14–18	–	[28]
Porphyridium cruentum	28–39	9–14	40–57	–	[30]
Prymnesium parvum	28–45	22–38	25–33	1–2	[30]
Scenedesmus dimorphus	8–18	16–40	21–52	3–6	[28]
Scenedesmus obliquus	50–56	12–14	10–17	3–6	[28]
Spirogyra sp.	6–20	11–21	33–64	–	[28]
Spirulina maxima	60–71	6–7	13–16	2–5	[28]
Spirulina platensis	46–63	4–9	8–14	2–5	[28]
Synechoccus sp.	63	11	15	5	[30]

Table 2. Chemical composition of different microalgae expressed on a dry matter basis (%).

maximum efficiency for the conversion of light to ATP is approximately 27%. However, only 42.3% of PAR can be utilized [35]. Furthermore, light intensity and light quality also play a key role in the growth rate of the cell [36].Inorganic and organic carbon represent one of the main sources of nutrition for microalgae. Different microalgae species can be maintained at various concentrations of CO_2 due to a mechanism called the carbon concentration mechanism (CCM), which accumulates inorganic carbon, concentrating it in the CO_2 in RuBisCO [37].

Microalgae can assimilate carbon through three routes: (i) direct absorption of CO_2 by the cell membrane; (ii) the use of bicarbonate by inducing the enzyme carbonic anhydrase and (iii) transport of HCO_3^- by the cell membrane. The enzyme carbonic anhydrase catalyzes the reaction converting HCO_3^- into CO_2, moreover RuBisCO uses CO_2 as the substrate on which it forms phosphoglycerate. Limitations to CO_2 production can occur, slowing the rate of reaction. Thus, carbonic anhydrase is a very efficient enzyme that can generate high concentrations of CO_2 [38–40].

4. Requirements of microalgae biorefineries

In addition to CO_2, other nutrients such as phosphorus, nitrogen and trace metals are important for the production of microalgae. These provides the necessary conditions for microalgae to carry out the metabolic reactions necessary for growth and so generate biomass or primary metabolites [41]. Most microalgae species can utilize inorganic and organic nitrogen sources.

Ammonium salts, ammonium sulfate, diammonium hydrogen phosphate and ammonia are supplied as inorganic nitrogen sources, and urea is supplied as an organic nitrogen source [42–43]. Phosphorus is supplied as hydrogen phosphate and dihydric phosphate in small amounts. Normally, sufficient quantities of minerals such as cobalt, copper, iron, molybdenum, manganese and zinc are present in the water supply, or they may be added as specific salts [44]. An adequate supply of nutrients is a prerequisite for high production rates. The introduction of certain nutrient stresses may affect the biochemistry. For example, nitrogen stress is important for carotenogenesis in *Dunaliella salina* [45] and increased lipid production in *Chlorella vulgaris* [46].

The most important factor in CO_2 fixation and microalgae biomass production is light intensity. Light sources can be divided into natural sunlight, which is applied in both open and closed cultivation, and artificial cold light that is mainly applied in closed cultivation. Various studies have been performed on the use of artificial light. Many lamps are available commercially such as light emitting diodes (LED), fluorescent tubes, halogen, tungsten, and high intensity discharge lamps (HID), and optical fibers. The investment costs, shelf life and stability of light intensity are important factors to consider when choosing a lighting source [47]. Recommended light sources for microalgae cultivation include the following: In laboratory research fluorescent tubes exhibiting a PAR efficiency of 1.25 μmol-ph s^{-1} W^{-1} are used, while HID with a PAR efficiency of 1.87 μmol-ph $s^{-1}W^{-1}$ are the most commonly employed in horticulture, along with LED lamps with a PAR efficiency of 1.91 μmol-phs$^{-1}W^{-1}$ [47]. The same type of lamp can emit different wavelengths, with blue LED and red LED having an adsorption at around 450–470 nm and 645–665 nm respectively [48]. Wang et al. [49] found the highest biomass yield using red LED in *Spirulina platensis* cultivation [49]. An analysis of Table 3 demonstrates a variety of possible sources of illumination for use in microalgae production.

The rate of photosynthesis is proportional to light intensity. When irradiance is increased, microalgal growth rate accelerates, but exposure of cells to long periods of high light intensity causes photoinhibition. Microalgae can only utilize the energy available in the 400–700 nm wavelength range, represented by PAR [50]. Moreover, several studies reported that the optimal wavelength varied from species to species [51]. Light intensities of 100 and 200 μE/m^2/s are frequently used [52].

Besides light intensity, it was found that light–dark cycles could also significantly influence microalgal growth by avoiding sustained exposure to high photon flux density and providing dark time for microalgae to repair photo-induced damage [53].

The light–dark periods for most microalgal cultivation are 24h:0h,16h:8h and 12h:12h. This varies with microalgal species. Experiments carried out with *Aphanothece microscopica Nägeli* in photoperiods of (22:2), showed that growth rate is not conditional on incident lighting over 22 h. This provides evidence that *Aphanothece microscopica Nägeli* have the possibility of storing energy for their biochemical processes, without affecting the rate of photosynthetic metabolism [54].

On the one hand, the light intensity of natural sunlight is cheaper, but the light cycle depends on weather and latitude, which often preclude higher biomass production. At the same time,

artificial illumination is generally expensive, but the control of light intensity afforded is excellent, allowing for greater flexibility and constant biomass production [55].

Light source	Commentary	Electrical consumption	Stability	Investment cost	Weather durability	Ref.
Conventional lamp (halogen, tungsten, fluorescent)	High productivity of biomass, large area lighting, generation of high temperature	High	High	Low	Moderate	[47]
LED lamp	Low heat generation, Greater resistance to on/off cycles	Low	High	Low	High	[47,56]
HID lamp	Generation of high temperature, high efficiency. However, losses from trapped light in protective covers and lenses, inefficient ballasts	Low	High	Low	Moderate	[47]
Optical fiber excited by lamps	Small space requirements for installation, good light distribution, uniformity of illumination, low risk of contamination	High	Moderate	High	High	[57]
Natural sunlight	Variable biomass productivity depending on weather conditions, good lighting area, economic and adequate light distribution	Absent	Low	Low	High	[55]

Table 3. Characteristics and power consumption for different sources of artificial light

A photobioreactor is a device consisting of an illuminated culture vessel designed for the controlled bioconversion of CO_2 into biomass and bioproducts. The two basic types of photobioreactors used for the large scale culture of microalgae are open or closed systems. Open systems can be built more easily, are more economical and relatively simple to control in relation to closed systems. Most open systems are natural lakes or open ponds. Two types of open systems are known: (i) circular ponds stirred with a rotating arm and (ii) raceway ponds, which are shallow artificial ponds divided into a rectangular grid with paddle wheels for culture mixing. Raceway ponds are the most popular open system design [58].

The reactor surface is illuminated with natural light and the intensity of illumination affects the microalgae culture. The depth of this type of reactor may not exceed 35–40 cm so that it does not prevent the passage of light to the bottom of reactor. The reactor performance declines with increasing depth due to the fact that diminishing amounts of light energy are available.

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Moreover, the use of an open system for the sole purpose of CO_2 sequestration is mitigated by the very low residence time of gas in the culture, which therefore offers a short time in which the fixing of CO_2 from flue gases by the microalgae can occur. Open systems produce low yields of products with high added value due to contamination problems [55]. Closed systems support high yields of microalgae biomass and they have certain advantages with regard to minimizing contamination, allowing axenic microalgal cultivation, providing a control system for various parameters such as pH, temperature, light, and CO_2 concentration. They also reduce CO_2 losses, prevent water evaporation, allow for a greater control of biomass growth, and permit the production of complex biomolecules. Closed systems are currently being assessed for microalgae cultivation in configurations such as flat-plate, vertical tubular, horizontal tubular and hybrid type photobioreactors [59].

Flat-plate and tubular photobioreactors are the commonest types used for cultivation of microalgae in the laboratory and on a pilot scale. These photobioreactors are based on the same principles of a large surface-to-volume (S/V) ratio, optimal use of CO_2 and suitable mixing. Tubular photobioreactors (airlift or bubble column) seem the most suitable for CO_2 sequestration due to their homogeneous mixture, greater gas transfer, smaller hydrodynamic stress, ease of construct and high productive output. Flat-plate photobioreactors are very expensive to build, which makes them unfeasible for industrial use. A hybrid photobioreactor is a combination of at least two types of different photobioreactor. Usually, integrating a horizontal tubular photobioreactor with a vertical tubular photobioreactor will compensate for the drawbacks in scale-up and the enhanced S/V ratio of vertical tubular photobioreactors. There are many configurations that have been studied, producing good results [55].

Photobioreactor development is perhaps one of the major steps that should be undertaken for the efficient large-scale cultivation of microalgae and bioproduct formation. Shape considerations must be taken into account when installing a system to produce bioproducts. Closed reactors are best for production of compounds of high added value.

There is a complex CO_2 transfer process in a photobioreactor. In the gas aerating method, mass transfer performance and biochemical reaction rate depend of the type and size of the photobioreactor, the range of operational conditions, the influence of physicochemical properties on hydrodynamics due to the high viscosity of the liquid, its rheological behavior, the measuring method used, bubble size, gas hold-up, the gas/liquid contact area, and CO_2 concentration and gas/liquid ratio [55].

In terms of solubility, oxygen is less soluble in water than CO_2. However, both gases are poorly soluble in aqueous solution so there is a need for the provision of these elements throughout the process. CO_2 bubbling in solution alone does not produce complete dissolution, since a fraction of the CO_2 injected is lost in the gas outlet. The chemical reactivity of CO_2 in the water forms H_2CO_3. The pH decreases with increasing insolubility and carbonic acid formation. The H_2CO_3 dissociates to HCO_3^- and CO_3^{2-}. Consequently, the total inorganic carbon concentration is represented by the totality of the compounds CO_3^{2-}, HCO_3^- and CO_2 [4].

The volumetric mass transfer coefficient (K_La) is the property of the photobioreactor that determines the appropriate conditions that will ensure cell growth in the reactor. K_La repre-

sents a function of microalgal characteristics and operating conditions. Efficiency of CO_2 transfer is necessary to increase the $K_L a$ of CO_2 allowing for improved transfer of gas to the liquid phase. Photobioreactors require an efficient CO_2 transfer system [4].

During microalgae growth in the photobioreactor, the accumulation of O_2 can occur. The water dissociation activity of PSII is responsible for the oxygen produced during photosynthesis. The increase of O_2 in the culture medium is a hard problem to solve. The level of dissolved O_2 in the culture medium causes photoinhibition reducing photosynthetic efficiency. Accumulation of O_2 becomes a complicated problem in a closed photobioreactor when the reactor configuration does not provide an interface between the culture and the surrounding atmosphere, in contrast to horizontal tubular reactors or a flat panel configuration. The solutions proposed to date rely on the installation of a degasser. In photobioreactors, degassing is only necessary when O_2 production (due to basal activity of PSII) is higher than respiration [60].

5. Microalgal biomass harvesting technologies

When the biochemical process in the photobioreactor have finished, the upstream processing ends and gives way to downstream processing and harvesting of the biomass and refining of the bioproducts in the biorefinery. As a result, most of the production costs in microalgae biorefineries are influenced by DSP. The microalgal harvest and reduction of its water content does not depend on a single method. Efficient and profitable harvesting methods are required to process the biomass and bioproducts economically [61]. The microalgae recovery techniques represent between 20–30% of total production costs. Of harvesting techniques, the most commonly used are flocculation, filtration, centrifugation, gravity sedimentation and flotation [62]. Some factors influencing the choice of harvesting technique are morphology, density and size of the microalgae, as well as the type and quality of product to be obtained [35].

Flocculation is a process in which particles are dispersed from the medium by using chemicals to aggregate the microalgal cells. Flocculants stimulate flocculation by causing colloids and other suspended particles in solution to form flocs. Chemical flocculants regularly used to harvest microalgae include ferric chloride, aluminum sulfate, alum, ferric sulfate, polyferric sulfate, as well as cationic polymers (polyelectrolytes), and organic flocculants (chitosan). Researchers have developed a process of cell autoflocculation, through the adjustment of pH in the microalgae culture [63].

Filtration operated under pressure or in a vacuum is satisfactory for recovering relatively large (>70mm) and/or filamentous microalgae, but is less effective in separating microalgae species with dimensions close to those of prokaryotes. Membrane microfiltration and ultrafiltration processes may be an option for the recovery of microalgae biomass under 30 mm [63]. Petrusevski [64] recovered 70–89% of microalgae using cross flow filtration with the advantage of maintaining the integrity of the microalgae biomass. On the other hand, use of a chamber-membrane filter press could achieve a concentration factor of 245 times the original concentration for *Coelastrum proboscideum* and produce a sludge with 27% solids [65]. Filtration is an expensive process due to membrane exchange and pumping. At larger scales of production

(>20 m^3 per day) other methods can be cheaper. For the processing of small volumes (e.g., < 2 m^3 per day) it can be more cost effective when compared with centrifugation [66].

Centrifugation is a methodology that includes the use of centrifugal acceleration for the sedimentation of microalgae in heterogeneous mixtures. The size and density of the structure determines the centrifugal separation of element. The supernatant is a liquid located in the upper layer of the centrifuge tube and the microalgae concentrate is represented by the remnant solid. The fast and intensive process depends on the sedimentation of cells for biomass recovery, as well as the amount of time of the cell suspension is in the centrifuge, and settling depth [63]. This method can lead to cell injury due to the gravitational and shear forces encountered [67]. Many researchers have recommended this method for the reliable recovery of microalgae [68–70]. Recovery by centrifugation is an efficient method when used with small volumes of fluid and high energy consumption. The drawbacks of centrifugation are the high initial investment costs, the noise generated during operation, and the cost of the electricity used [71].

Gravitational sedimentation is widely used to separate microalgae in aqueous solution and for wastewater treatment. The sedimentation rates of microalgae are influenced by the rate of sedimentation of solids and are determined by the density and area of the microalgae cells [35]. Gravitational sedimentation, preceded by flocculation, is one of the most widely-used techniques for the harvesting of microalgae biomass. Disadvantages of the method are that is very slow (0.1 to 2.6 m h^{-1}) and the biomass may suffer decomposition under conditions of high temperature. Furthermore, the technique is suited for use with large microalgae or those with a filamentous morphology [72].

Flotation methods are based on the binding of microalgae cells using air bubbles. The resulting flocs rise to the surface of the liquid and are recovered by either physical or chemical procedures. Particles as small as 500 μm can be recovered by flotation. Some strains have gas vacuoles and float at the surface of the water [72]. The incorporation of air bubbles depends on several aspects such as the contact angle of air, solid, and aqueous phases. According to the method of bubble production, flotation techniques can be divided into dissolved air flotation (DAF), dispersed flotation and electrolytic flotation. DAF is the most used method in the treatment of industrial wastewater. Microalgae cells are recovered by dissolving air in the water under pressure and are then released into a reservoir at atmospheric pressure thus producing small bubbles. Chemical flocculation has been used with DAF to separate microalgae [73]. Garg et al. [74] evaluated the effects of froth flotation in different microalgae strains and found that *Chlorella* sp. showed a good response of floatability due to its high hydrophobicity. This method represents a promising choice for the industrial scale harvesting of microalgae and represents a very versatile technique for the separation of small particles. Microalgae with low surface hydrophobicity are difficult to harvest by flotation separation. Surface hydrophobicity and bubble size are the key factors affecting algae flotation, and a stepwise optimization can lead to effective separation by flotation of difficult-to-harvest microalgae [64]. Although flotation has been widely used by researchers as a harvesting method, there are feasibility and economic limitations.

6. Biorefinery

Biorefinery comprises a number of specialized methods used to extract the most out of primary and/or secondary metabolic products. Microalgae biorefineries must use methods and technology for isolating compounds and obtaining principal constituents from biomass, without damaging one or more of the product fractions, thereby adding value to the bioproduct formed [75].

The main focus when obtaining the products should be the dehydration or drying of the biomass when there is a requirement for its immediate use, otherwise the harvested biomass suspension must be processed rapidly. Methods used for drying microalgae include lyophilization, spray drying, drum drying and sun drying. The next step is cell disruption as some target products are intracellular and therefore cell disruption is required in order to release the products and ready them for extraction. Several methods can be used depending on the metabolites of interest [76].

The product fractions obtained from microalgae can be transformed into high-value molecules, antioxidants, anti-inflammatories, natural pigments, biofuels, and food supplements for human and animal feed. Microalgae biorefinery is, therefore, a process of great industrial impact and must be undertaken properly (Figure 2).

Methods of drying microalgal biomass are used with the purpose of increasing the longevity of cells. Drying methods may include sun drying, lyophilization, drum drying, spray drying, and fluidized bed drying. Sun drying is cheap but is very slow. Spray drying is the method chosen for obtaining bioproducts with a high added value, though this procedure is not recommended for extraction of microalgae pigments as it may affect the pigments' molecular structure. Lyophilization is widely used in scientific research procedures, but is very expensive for use on a large scale. It is useful with respect to some enzymes and pharmaceuticals. This method eliminates thermal and osmotic damage and preserves the cell constituents microalgae [77].

By comparing different drying techniques (sun drying, lyophilization) for the effective extraction of lipids from *Scenedesmus* sp. grown in a raceway reactor Guldhe et al. [77] showed that drying methods are critical for effective downstream processing in the synthesis of microalgal biodiesel. No statistically significant difference was found in the drying methods used for the extraction of lipids

The extraction of intracellular components requires the breakdown of the microalgal cell wall. Various disruption methods involving chemical treatments (solvents, acids), mechanical treatments (ultrasound, high-pressure homogenization, bead beating and blending), autoclaving, freezing–thawing sequences and supercritical fluids have been used. Some of the extraction and fractionation techniques will be described briefly below. Microalgae biorefineries seek to apply these methods at an industrial scale and at low cost. Their high functionality with low concentrated streams is advantageous. Choosing the most appropriate method depends of various biological factors and the energy required. The integration of cell disrup-

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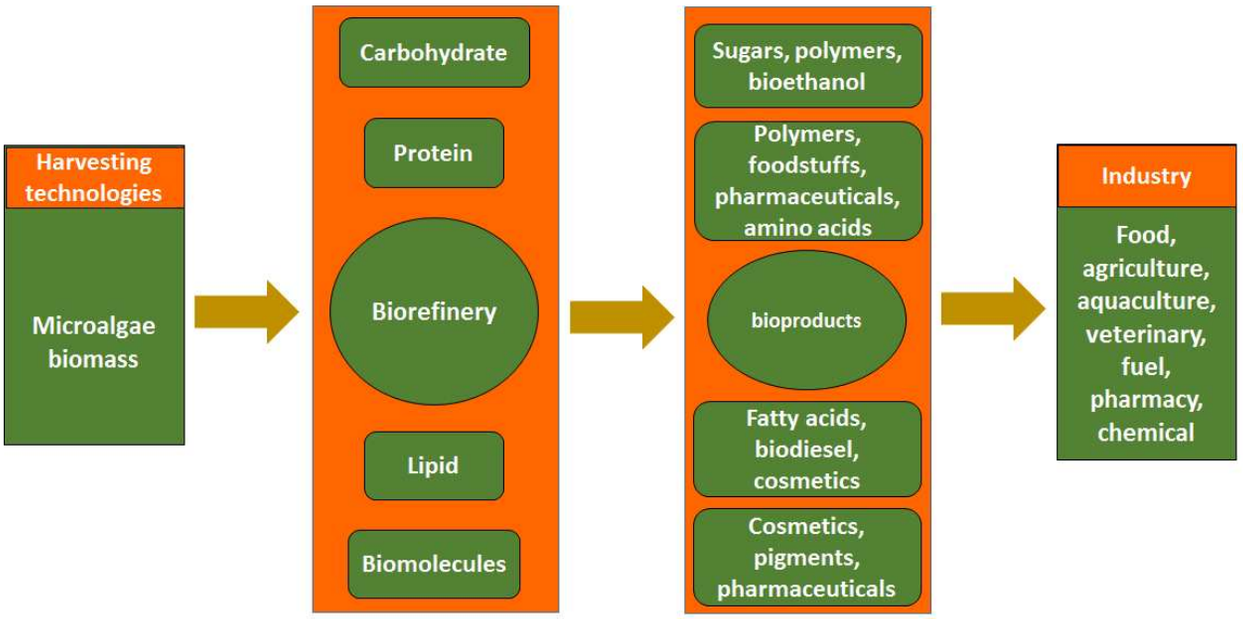

Figure 2. General outline of microalgae biorefinery.

tion into downstream processing has to be easy and should not have a negative impact on subsequent processing steps [78].

Pulsed electric field procedure can be a promising alternative to conventional cell disintegration methods. The procedure is based on the dosing of short electrical pulses in high intensity electric fields. These alter the structure of the cell membrane, which as a result loses its barrier function and becomes permeable — a phenomenon often referred to as electroporation [79].

Goettel et al. [78] evaluated the application of the pulsed electric field procedure for the disruption of *Auxenochlorella protothecoides* cells. For all pulse parameters applied, there was evidence that cell disintegration resulted in the release of soluble intracellular matter into the suspension. The efficiency of cell disruption improved with increasing treatment energy, whereas the field strength had no major influence. Thus, the investigation proposed the use of the pulsed electric field procedure of cell disruption and selective two-step extraction. As an initial step, the pulsed electric field procedure allows separation of water soluble intracellular substances. In a subsequent step, lipids can be very efficiently extracted by solvents.

The ultrasound method is based on the incorporation of high frequency sound waves in microalgae cells so that pressure variation can disrupt the cell wall [80].

Ehimen et al. [81] successfully used ultrasound to improve methods of oil extraction in *Chlorella* biomass samples. The results of the study showed that it is feasible to reduce volumes of methanol used in the trans-esterification process. The combination of an ultrasonic process and solvent use demonstrated the potential for recovery of greater yields of fatty acid methyl esters (FAME) for biodiesel production.

Enzymatic degradation is another method of cell wall disruption. This method is used on a laboratory scale since high costs limits its use on a larger, industrial scale. The advantage is

that the enzyme may be inactivated, removed, recovered and reused. It has a high specificity without interfering in the recovery of bioproducts. Enzymatic disruption of microalgae cell walls can be performed with a mixture of β-glucanases and lysozyme. Studies of enzymatic hydrolysis of *Chlorella* cell walls have demonstrated the high specificity of the disruption so that mechanical degradation can be performed with low energy costs [82].

Chemical treatments using acid are performed by immersing diluted microalgae biomass in strong acid followed by a strong base, at high temperature for a specific time. One disadvantage of the method is the toxicity of the acid, and as a result the method is not widely used [83]. Sathish and Sims [83] demonstrated a method of extracting transesterifiable lipids using acid and base hydrolysis for *Chlorella* sp. and *Scenedesmus* sp. with 84% of moisture. On average, 60% of lipids were extracted and converted to biodiesel by transesterification. This was achieved without drying the recovered biomass and the use of a smaller volume of organic solvent was evident.

The choice of cell disruption method is dependent on the bioproduct, the strain of microalgae used, and the costs and efficiency of the process

7. Extraction and purification of microalgae metabolites

Solvent extraction systems are extensively used to extract microalgae metabolites from processed biomass. Solvents such as ethanol, chloroform, diethyl ether, hexane and methanol are commonly used. These can extract carbohydrates, amino acids, salts, hydrophobic proteins, lipids and pigments. The disadvantages of solvent extraction are that: (i) the process requires high capital investments; (ii) the energy requirements are high; (iii) the solvent is highly flammable; and (iv) the difficulty of recovering the solvent [84].

Different process for the extraction of fatty acid from *Aphanothece microscopica Nageli*, *Phaeodactylum tricornutum*, *Isochrysis galbana* have been described [29,85]. Extraction using aqueous buffers is employed to obtain phycobiliproteins from *P. cruentum* and lutein from three *Chlorella* species [86].

The regeneration of solvent for subsequent operation is difficult, further decreasing the efficiency of extraction. A method that can recover the solvent for reuse would be ideal from an economic point of view. This phase splitting could be induced by changing the nature of the solvent [87].

Du et al [87] studied the extraction of oil from *Desmodesmus* sp. by CO_2-switchable solvents. In this research, the secondary amines dipropylamine and ethylbutylamine were able to extract lipid from a liquid medium without damaging microalgae cells. These solvents allow the process of quick and efficient lipid extraction in the presence of water induced by the presence of ambient temperatures and atmospheric CO_2. These solvent systems provide a potential for reuse and recovery leading to decreased costs and provide an efficient method of microalgae lipid extraction [87]. Properties of the cell membrane are of great importance in the solvent extraction process. Therefore, disruption of the cell wall is critical [88].

Crude extracts are generally filtered and purified by several chromatographic methods in order to obtain the metabolite of interest. In choosing a chromatographic technique certain considerations should kept in mind. These include molecular weight, isoelectric point, hydrophobicity and biological affinity. Supercritical fluid extraction has been shown to be an efficient technique for extracting carotenoids from microalgae *Scenedesmus* sp. [89] and fatty acids, and of the three microalgae strains evaluated, *S. obliquus* is the best source of α-linolenic acid [90].

Some other chromatographic methods included reverse phase chromatography, silica gel adsorption chromatography, and ion exchange chromatography (for proteins). Chromatographic techniques are usually employed for higher-value products. An economical evaluation could be useful to help calculate the optimum conditions for industrial applications [91].

8. Potential uses for bioproducts obtained from microalgal biorefineries

Microalgae have massive potential to produce biomolecules due to the low cost of energy and nutrient sources used, as well as fast growth rates and the capacity to accumulate or secrete metabolites. Microalgal biorefineries allow the transformation of biomass into the production of fuels, food, feed, chemicals, polymers and value-added ingredients [92].

Thus, the use of these microorganisms in carbon sequestration processes combines the treatment of polluting compounds with the production of consumables in a variety of forms. Table 4 shows some potential uses for the bioproducts obtained from microalgal biorefineries and formation by the biological conversion of CO_2 in photobioreactors.

Activity	Application	Reference
Nutraceutical, antimicrobial, anti-inflammatory	Nutritional supplement, antiproliferative, combat infections and diseases.	[93,94]
Antioxidant, natural pigment	Supplement and food ingredient for humans, feeding of fish and shellfish.	[93,95]
Biofuels	Natural gas production in fermenters by the digestion of biomass for obtain biodiesel.	[96,97]
Fertilizers	Use of the biomass as a source of nitrogen and phosphorous in tillable land.	[98]
High-value molecules	Chlorophyll-*a*, phycocyanin, β-carotene, γ-linolenic acid, eicosapentaenoic acid and stable biochemical isotopes.	[99]
Anticancer and antitumor	Antiproliferative. Inducing G1 inhibition in post-gastric carcinoma cells.	[94]
Chemical industry	Volatile organic compounds.	[100]

Table 4. Potential uses for bioproducts obtained from microalgal biorefineries

Microalgae possess a versatile metabolic capacity that can be transformed into valuable products through various processing routes. Some microalgae species as *Chlorella*, *Chlamydomonas*, *Dunaliella*, *Scenedesmus*, and *Tetraselmis* have a high carbohydrate content (37–55%) that mainly comes from starch in chloroplasts and cellulose cell walls [101]. Carbohydrate-rich microalgal biomass were evaluated for bioethanol production and were found to provide good yields [102].

The lipid profile of microalgae shows values of 2–77% depending on species and growth conditions. Microalgae lipids are classified into two groups, one for transformation in biofuel and one for food supplements, with carbon numbers of between 14–20 and 20 carbons respectively. Microalgae have a promising future, with production of eicosapentaenoic acid and docosahexaenoic acid as the main product. The species of microalgae producing omega-3 polyunsaturated fatty acids are mainly *Bacillariophyta, Chlorophyta, Cryptophyta, Haptophyta, Heterokontophyta* and *Rhodophyta* [103,104].

Proteins are among the main constituents of microalgae, at proportions of 50–70% depending on species, and they are an important product of microalgae biorefineries. Microalgal proteins can be used in human or animal nutrition (from aquaculture to farm animals). However, some microalgae contain toxic proteins, so analytical analyses need to be performed [105]. Nutritional and toxicological evaluations have demonstrated that microalgal biomass offers a valuable feed supplement or substitute for conventional animal feed sources [106].

Microalgae are known to be a good source of pigments and bioactive compounds. Chlorophylls, phycobilins and carotenoids are molecules with a high added value that can be obtained from *Porphyridium cruentum, Synechococcus* sp. and *Chlorella* and used in the chemical industry. Rodrigues et al. [107] showed that *Phormidium autumnale* has potential for the production of carotenoids. Sensitivity analysis showed the possibility of obtaining 107,902.5 kg/year of total carotenoids at the industrial scale. Symplostatin and curacin A have been isolated from the cyanobacteria *Symploca hydnoides* and *Lyngbya majuscula* respectively. These compounds exhibited cytotoxicity against a human carcinoma cell line [108].

The microalgae biorefineries industry promises much from the economic point of view. Global annual sales of beta-1,3-glucan from *Chlorella* sp. are in excess of USD$38 billion [105]. Moreover, phycobiliproteins present in cyanobacteria and some algae used to develop compounds for the pharmaceutical industry, represented a market of about USD$6–11 million with prices that varied from USD$3–25 mg^{-1} [105]. Considering that Kenekar and Deodhar [109] reported a phycocyanin yield of 0.071 gL^{-1} in *Geitlerinema sulphureum* culture, a photobioreactor with 100 L could generate a profit of approximately USD$177,500 [109]. Microalgae biomass produces more than 5,000 tons of dried mass/year with an annual revenue greater than USD$1.25 billion, not including processed products, demonstrating the potential of this type of biotechnological process [99]. Despite the promising conditions for the production of microalgae biomass and bioproducts, the industrial-scale development is currently a long way from the high profits available in theory. This is due to the lack of methods and photobioreactors that can produce large enough quantities to supply the market [12].

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Finally, microalgae cells can produce methane. Sialve et al. [110] showed methane production values from anaerobic digestion in microalgae biomass in the range of 0.09–0.54 L CH_4/g volatile solids [110]. Furthermore, compounds such as non-methane hydrocarbon, organohalogens, and aldehydes are continuously being formed and released from the liquid phase of photobioreactors. The production of renewable polymers is an emerging industrial field [4].

Therefore, microalgal biotechnology can be seen as a promising scientific tool in the near future and microalgae biorefineries have the potential to solve some of the environmental, nutritional and pharmaceutical problems afflicting society.

9. Final considerations

Most research into microalgae biorefineries has been undertaken at the laboratory or pilot scale, and the number of full-scale studies is limited. Large-scale microalgae processes have been developed mainly using open photobioreactors. Some successful initiatives have been carried out in closed systems, but the closed systems need to operate at a large scale, to overcome the many drawbacks. At a large scale, algal growth conditions need to be closely controlled. The processes can be economical when using inexpensive sources of CO_2 from flue gas emissions, wastewaters, and/or with the extraction of bioproducts for industrial use.

Finally, many companies are investing in biotechnology, increasing spending on the production systems in order to obtain microalgal biofuel and high value-added bioproducts. Although at present there is no consensus on the criteria for the large scale development of photobioreactors for microalgae cultivation. Conventional configurations of closed systems, and hybrid photobioreactors are being employed and constantly improved for use at the industrial scale.

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