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\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{2} in the atmosphere. During the twentieth century an increase in CO\textsubscript{2} concentration of 30% was observed. This rate of increase
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 sulfuric 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 microalgal 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 CO₂ capture from winegrowing for production of oil, nutraceuticals, food additives and biochemical compounds.                                                 | [25]|  
| IGV Biotech              | Nuthetal, Germany | Founded in 1960. IGV Biotech develops microalgal 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 CO₂ capture. | [26]|  
| Algenol Biofuels         | Florida, USA | Founded in 2006. The company uses CO₂ 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 CO₂ sequestering technology for algae culture.
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 microalgal 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$_2$O), O$_2$ and H$_2$O 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$_2$O. The theoretical
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 provide 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.

| Microalgal species       | Protein  | Lipid | Carbohydrate | Nucleic acid | Ref.  |
|--------------------------|----------|-------|--------------|--------------|-------|
| Anabaena cylindrical     | 43–56    | 4–7   | 25–30        | –            | [28]  |
| Aphanothece microscopica | 41–49    | 8–9   | 13–18        | 3–4          | [29]  |
| Nügeli                    |          |       |              |              |       |
| 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 bicornulata    | 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]  |
| Synechococcus sp.        | 63       | 11    | 15           | 5            | [30]  |

Table 2. Chemical composition of different microalgae expressed on a dry matter basis (%).
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 *Aphanathece microscopica Nägeli* in photoperiods of (22:2), showed that growth rate is not conditional on incident lighting over 22 h. This provides evidence that *Aphanathece 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, | High productivity of biomass, large area lighting, generation of high       | High                   | High      | Low             | Moderate           | [47] |
| tungsten, fluorescent)       | temperature                                                                  |                        |           |                 |                    |      |
| LED lamp                     | Low heat generation, Greater resistance to on/off cycles                    | Low                    | High      | Low             | High               | [47,56] |
|                              | Generation of high temperature, high efficiency.                            |                        |           |                 |                    |      |
| HID lamp                     | However, losses from trapped light in protective covers and lenses, inefficient ballasts | Low                    | High      | Low             | Moderate           | [47] |
| Optical fiber excited by     | Small space requirements for installation, good light distribution, uniformity of illumination, low risk of contaminated light | High                   | Moderate  | High            | High               | [57] |
| lamps                        |                                                                            |                        |           |                 |                    |      |
| 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 CO₂ 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.
Moreover, the use of an open system for the sole purpose of CO₂ 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 CO₂ 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 CO₂ concentration. They also reduce CO₂ 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 microalgal 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 CO₂ and suitable mixing. Tubular photobioreactors (airlift or bubble column) seem the most suitable for CO₂ 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 CO₂ transfer process in a photobioreactor. In the gas aeration 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 CO₂ concentration and gas/liquid ratio [55].

In terms of solubility, oxygen is less soluble in water than CO₂. However, both gases are poorly soluble in aqueous solution so there is a need for the provision of these elements throughout the process. CO₂ bubbling in solution alone does not produce complete dissolution, since a fraction of the CO₂ injected is lost in the gas outlet. The chemical reactivity of CO₂ in the water forms H₂CO₃. The pH decreases with increasing insolubility and carbonic acid formation. The H₂CO₃ dissociates to HCO₃⁻ and CO₂²⁻. Consequently, the total inorganic carbon concentration is represented by the totality of the compounds CO₂²⁻, HCO₃⁻ and CO₂ [4].

The volumetric mass transfer coefficient (K₁,a) is the property of the photobioreactor that determines the appropriate conditions that will ensure cell growth in the reactor. K₁,a repre-
sents a function of microalgal characteristics and operating conditions. Efficiency of CO₂ transfer is necessary to increase the Kₜ,a of CO₂ allowing for improved transfer of gas to the liquid phase. Photobioreactors require an efficient CO₂ transfer system [4].

During microalgae growth in the photobioreactor, the accumulation of O₂ can occur. The water dissociation activity of PSII is responsible for the oxygen produced during photosynthesis. The increase of O₂ in the culture medium is a hard problem to solve. The level of dissolved O₂ in the culture medium causes photoinhibition reducing photosynthetic efficiency. Accumulation of O₂ 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 O₂ 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]. Petrucevski [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 m³ per day) other methods can be cheaper. For the processing of small volumes (e.g., < 2 m³ 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 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 on various biological factors and the energy required. The integration of cell disrup-
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 microalgal 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 microalgal 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 microalgal metabolites

Solvent extraction systems are extensively used to extract microalgal 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 CO₂-switchable solvents. In this research, the secondary amines dipropylamine and ethylbutylamine were able to extract lipid from a liquid medium without damaging microalgal 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 CO₂. These solvent systems provide a potential for reuse and recovery leading to decreased costs and provide an efficient method of microalgal 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 *Symplioca 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]. Microalgal biomass produces more than 5,000 tons of dried mass/year with an annual revenue greater than USD$1.25 billion, not including processed products, demonstrating the potential of this type of biotechnological process [99]. Despite the promising conditions for the production of microalgae biomass and bioproducts, the industrial-scale development is currently a long way from the high profits available in theory. This is due to the lack of methods and photobioreactors that can produce large enough quantities to supply the market [12].
Finally, microalgae cells can produce methane. Sialve et al. [110] showed methane production values from anaerobic digestion in microalgae biomass in the range of 0.09–0.54 L CH$_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.

Author details

Eduardo Jacob-Lopes*, Luis Guillermo Ramírez Mérida, Maria Isabel Queiroz and Leila Q. Zepka

*Address all correspondence to: jacoblopes@pq.cnpq.br

1 Food Science and Technology Department, Federal University of Santa Maria, UFSM, Santa Maria, RS, Brazil

2 Department of Biology, University of Carabobo, UC, Avenue Universidad, Valencia, Edo. Carabobo, Venezuela

3 School of Chemistry and Food, Federal University of Rio Grande, FURG, Eng. Alfredo Huch, CEP Rio Grande, RS, Brazil
References

[1] Song C. Global challenges and strategies for control, conversion and utilization of CO₂ for sustainable development involving energy, catalysis, adsorption and chemical processing. Catalysis Today 2006; 115(1–4):2-32.

[2] United Nations. Kyoto Protocol to the United Nations Framework Convention on Climate Change. 1998. http://unfccc.int/resource/docs/convkp/kpeng.pdf. (accessed 28 March 2014).

[3] Ramírez-Mérida L., Zepka Q.L., Jacob-Lopes E. Microalgae and cyanobacteria: application in medicine. Revista Electrónica PortalesMedicos.com 2014; 9(4):149.

[4] Jacob-Lopes E., Teixeira Franco T. Microalgae-based Systems for Carbon Dioxide Sequestration and Industrial Biorefineries. In: Momba M. and Bux F. (ed.) Biomass. Croatia: Sciyo; 2010. p135-146.

[5] Vanthoor-Koopmans M., Wijffels R., Barbosa M., Eppink M. Biorefinery of microalgae for food and fuel. Bioresource Technology 2013; (135):142-149.

[6] Singh R.M., Sharma S. Development of suitable photobioreactor for algae production – A review. Renewable & Sustainable Energy Reviews. 2012;16(4):2347-2353.

[7] Ramírez-Mérida L., Zepka Q.L., Jacob-Lopes E. Photobioreactor: tool for mass cultivation of cyanobacteria. Ciencia y Tecnología 2013; 6(2):9-19.

[8] Thitakamol B., Veawab A., Aroonwilas A. Environmental impacts of absorption based CO₂ capture unit for post-combustion treatment of flue gas from coal-fired power plant. International Journal of Greenhouse Gas Control 2007; 1:318-342.

[9] Le Quéré C., Raupach M.R., Canadell J.G., Marland G., Bopp L., Ciais P., Conway T.J., Doney S.C., Feely R.A., Foster P., Friedlingstein P., Gurney K., Houghton R.A., House J.I., Huntingford C., Levy P.E., Lomas M.R., Majkut J., Metzl N., Ometto J.P., Peters G.P., Prentice I.C., Randerson J.T., Running S.W., Sarmiento J.L., Schuster U., Sitch S., Takahashi T., Viovy N., van der Werf G.R., Woodward F.I. Trends in the sources and sinks of carbon dioxide. Nature Geoscience 2009; 2:831–6.

[10] Kadam K.L. Power plant flue gas as a source of CO₂ for Microalgae cultivation: economic impact of different process options. Energy Conversion and Management 1997; 38(Suppl.):505–10.

[11] Ho S-H., Li P-J., Liu C-C., Chang J-S. Bioprocess development on microalgae-based CO₂ fixation and bioethanol production using Scenedesmus obliquus CNW-N. Bioresource Technology 2013, (145):142-149.

[12] Ramírez-Mérida, L.G., Zepka, L.Q., Jacob-Lopes, E. Why does the Photobioreactors Fail? Journal of Bioprocessing & Biotechniques 2015; 5:7.
[13] Jacob-Lopes, E., Revah, S., Hernández, S., Shirai, K., Franco, T.T., 2009. Development of operational strategies to remove carbon dioxide in photobioreactors. Chemical Engineering Journal 153, 120-126.

[14] Jacob-Lopes, E., Lacerda, L.M.C.F., Franco, T.T., 2008. Biomass production and carbon dioxide fixation by Aphanothece microscopica Nägeli in a bubble column photobioreactor. Biochem. Eng. J. 40, 27-34.

[15] Skjanes K, Lindblad P, Muller J. BiOCO$_2$ — a multidisciplinary, biological approach using solar energy to capture CO$_2$ while producing H$_2$ and high value products. Biomolecular Engineering 2007; 24:405–13.

[16] Wang B., Li Y.Q., Wu N., Lan C.Q. CO$_2$ bio-mitigation using microalgae. Applied Microbiology and Biotechnology 2008; 79:707–718.

[17] Van Den Hende S., Vervaeren H., Boon, N. Flue gas compounds and microalgae: (bio-) chemical interactions leading to biotechnological opportunities. Biotechnology Advances 2012; 6:1405–1424.

[18] Kumar K, Dasgupta C.N., Nayak B., Lindblad P., Das D. Development of suitable photobioreactors for CO$_2$ sequestration addressing global warming using green algae and cyanobacteria. Bioresource Technology 2011; 102:4945–4953.

[19] Kvamsdal H.M., Haugen G., Svendsen H.F. Flue-gas cooling in postcombustion capture plants. Chemical Engineering Research and Design 2011; 89:1544–1552.

[20] Chiu S.Y., Kao C.Y., Huang T.T., Lin C.J., Ong S.C., Chen C.D., Chang J.S., Lin C.S. Microalgal biomass production and on-site bioremediation of carbon dioxide, nitrogen oxide and sulfur dioxide from flue gas using Chlorella sp. cultures. Bioresource Technology 2011; 102:9135–9142.

[21] Xu Y., Isom L., Hanna M.A. Adding value to carbon dioxide from ethanol fermentations. Bioresource Technology 2010; 101:3311–3319.

[22] Algae Floating Systems technologies. AFS BioOil. http://www.afsbiioil.com/company-history.html (accessed 18 August 2014).

[23] Algae Floating Systems, Inc. AFS Biofarm™. http://www.algaefloatingsystems.com/company-3.html (accessed 18 August 2014).

[24] Seambiotic Ltd. http://www.seambiotic.com/home-2/ (accessed 18 August 2014).

[25] Aeon Biogroup. http://www.aeonbiogroup.com/ (accessed 18 August 2014).

[26] IGV Biotech. http://www.igv-gmbh.de/en/home/ (accessed 18 August 2014).

[27] Algenol Biofuels. http://www.algenol.com/ (accessed 18 August 2014).

[28] Becker E.W. Microalgae as a source of protein. Biotechnology Advances 2007; 25:207–210.
[29] Zepka Q.L., Jacob-Lopes E., Goldbeck R., Queiroz M.I. Production and biochemical profile of the microalgae *Aphanothece microscopica* Nageli submitted to different drying conditions Chemical Engineering and Processing 2008; 47:1305–1310.

[30] Zhu L.D., Hiltunen E., Antila E., Zhong J.J, Yuan Z.H., Wang Z.M. Microalgal biofuels: Flexible bioenergies for sustainable development. Renewable and Sustainable Energy Reviews 2014; 30:1035–1046.

[31] Bergman L., Ran D.G.A. Cyanobacterial–plant symbioses: signalling and development. In: Herrero A., Flores E. (ed.) The Cyanobacteria, Molecular Biology, Genomics and Evolution. Norfolk, UK: Caister Academic Press; 2008. p447–473.

[32] Nelson D.L., Cox M.M. Lehninger principles of biochemistry. (4 ed.) New Jersey: W.H. Freeman & Co.; 2005.

[33] Calvin M. 40 years of photosynthesis and related activities. Photosynthesis Research 1989; 21:3-16.

[34] Iverson T.M. Evolution and unique bioenergetic mechanisms in oxygenic photosynthesis. Current Opinion in Chemical Biology 2006; 10:91-100.

[35] Brennan L., Owende P. Biofuels from microalgae – a review of technologies for production, processing, and extractions of biofuels and co-products. Renewable & Sustainable Energy Reviews 2010; 14:557–577.

[36] Ravelonandro P.H., Ratianarivo D.H., Joannis-Cassan C., Isambert A., Raherimanidimby M. Influence of light quality and intensity in the cultivation of *Spirulina platensis* from Toliara (Madagascar) in a closed system. Journal of Chemical Technology and Biotechnology 2008; 83:842–848.

[37] Jaiswal P., Kashyap A. Isolation and characterization of mutants of two diazotrophic cyanobacteria tolerant to high concentrations of inorganic carbon. Microbiological Research 2002; 157:83-91.

[38] Marcus Y. Distribution of inorganic carbon among its component species in cyanobacteria: Do cyanobacteria in fact actively accumulate inorganic carbon? Journal of Theoretical Biology 1997; 185:31-45.

[39] Badger M.R., Price G.D. CO$_2$ concentration mechanisms in cyanobacteria: Molecular components, their diversity and evolution. Journal of Experimental Botany 2003; 54(383):609-622.

[40] Cuaresma M., Garbayo I., Vega J.M., Vilchez C. Growth and photosynthetic utilization of inorganic carbon of the microalga *Chlamydomonas acidophila* isolated from Tin-to river. Enzyme and Microbial Technology 2006; 40:158-162.

[41] Kumar A., Ergas S., Yuan X., Sahu A., Zhang Q., Dewulf J., Malcata F.X., van Langenhove H. Enhanced CO$_2$ fixation and biofuel production via microalgae: recent developments and future directions. Trends in Biotechnology 2010; 28:371–80.
[42] Welsh D.T., Bartoli M., Nizzoli D., Castaldelli G., Riou S.A., Viaroli P. Denitrification, nitrogen fixation, community primary productivity and inorganic-N and oxygen fluxes in an intertidal *Zostera noltii* meadow. Marine Ecology Progress Series 2000; 208:65–77.

[43] Hsieh C-H., Wu W-T. Cultivation of microalgae for oil production with a cultivation strategy of urea limitation. Bioresource Technology 2009; 100(17):3921–3926.

[44] Çelekli A., Yavuzatmaca M., Bozkurt H. Modeling of biomass production by *Spirulina platensis* as function of phosphate concentrations and pH regimes. Bioresource Technology 2009; 100(14):3625–3629.

[45] Ben-Amotz A., Avron A. The biotechnology of mass culturing *Dunaliella* for products of commercial interest. In: Cresswell R.C., Rees T.A.V., Shah N. (ed.) Algal cyanobacterial biotechnology. Essex: Longman Scientific & Technical; 1989 p91–114.

[46] Stephenson A.L., Dennis J.S., Howe C.J., Scott S.A., Smith A.G. Influence of nitrogen-limitation regime on the production by *Chlorella vulgaris* of lipids for biodiesel feedstocks. Biofuels 2010; 1:47–58.

[47] Blanken W., Cuaresma M., Wijffels R., Janssen M. Cultivation of microalgae on artificial light comes at a cost. Algal Research 2013; 2:333–340

[48] Yeh N.C., Chung J.P. High-brightness LEDs-energy efficient lighting sources and their potential in indoor plant cultivation. Renewable & Sustainable Energy Reviews 2009; 13:2175–2180

[49] Wang C.Y., Fu C.C., Liu Y.C. Effects of using light-emitting diodes on the cultivation of *Spirulina platensis*. Biochemical Engineering Journal 2007; 37:21–25.

[50] Melnicki M., Pinchuk G., Hill E., Kucek L., Stolyar S., Fredrickson J., Konopka A., Beliaev A. Feedback-controlled LED photobioreactor for photophysiological studies of cyanobacteria. Bioresource Technology 2013; 134:127–133.

[51] Katsoyannis T., Shimahara K., Shiraishi H., Yamagami K., Ranjbar R., Katoh S. Effect of flashing light from blue light emitting diodes on cell growth and astaxanthin production of *Haematococcus pluvialis*. Journal of Bioscience and Bioengineering 2006; 102:442–446.

[52] Zhao B., Su Y. Process effect of microalgal-carbon dioxide fixation and biomass production: A review. Renewable and Sustainable Energy Reviews 2014; 31:121–132.

[53] Janssen M., Tramper J., Mur L.R., Wijffels R.H. Enclosed out door photobioreactors: light regime, photosynthetic efficiency, scale-up, and future prospects. Bioengineering and Biotechnology 2003; 81:193–210.

[54] Jacob-Lopes E., Gimenes Scoparo C.H., Ferreira Lacerda L.C.M., Teixeira Franco T. Effect of light cycles (night/day) on CO₂ fixation and biomass production by microalgae in photobioreactors. Chemical Engineering and Processing 2009; 48:306–310.
Carvalho A.P., Meireles L.A., Malcata F.X. Microalgal reactors: a review of enclosed system designs and performances. Biotechnology Progress 2006; 22:1490–1506.

Schulze P.S.C., Barreira L.A., Pereira H.G.C., Perales J.A., Varela J.C.S. Light emitting diodes (LEDs) applied to microalgal production. Trends in Biotechnology 2014; 32(8): 422-430.

Xue S., Zhang Q., Wu X, Yan C, Cong W. A novel photobioreactor structure using optical fibers as inner light source to fulfill flashing light effects of microalgae. Bioresource Technology 2013; 138:141–147

Jacob-Lopes E., Zepka L.Q., Queiroz M.I. Cyanobacteria and carbon sequestration. In: Sharma N., Rai A and Stal L. (ed.) Cyanobacteria an economic perspective. Chichester:Wiley Blackwell; 2014. p65-71.

Singh R.M., Sharma S. Development of suitable photobioreactor for algae production – A review. Renewable & Sustainable Energy Reviews 2012; 16(4):2347–2353.

Dasgupta C.N., Gilbert J.J., Lindblad P., Heidorn T., Borgvang S.A., Skjanes K., Das D. Recent trends on the development of photobiological processes and photobioreactors for the improvement of hydrogen production. International Journal of Hydrogen Energy 2010; 35(19):10218–10238.

Wang B., Li Y., Wu N., Lan C. CO₂ bio-mitigation using microalgae. Applied Microbiology and Biotechnology 2008; 79(5):707–18.

Gudin C., Therpenier C. Bioconversion of solar energy into organic chemicals by microalgae. Advances in Biotechnological Processes 1986; 6:73–110.

Molina Grima E., Belarbi E.H., Acín Fernández F.G., Robles Medina A., Chisti Y. Recovery of microalgal biomass and metabolites: process options and economics. Biotechnology Advances 2003; 20(7–8):491–515.

Petruševaskis B., Bolier G., Van Breemen A.N., Alaerts G.J. Tangential flow filtration: a method to concentrate freshwater algae. Water Research 1995; 29:1419–1424.

Mohn F.H. Experiences and strategies in the recovery of biomass in mass culture of microalgae. In: Shelef G., Soeder C.J. (ed.) Algal biomass. Amsterdam: Elsevier; 1980. p547–71.

MacKay D., Salusbury T. Choosing between centrifugation and cross flow microfiltration. Chemical Engineering Journal 1988; 477:45–50.

Knuckey R.M., Brown M.R., Robert R., Frampton D.M.F. Production of microalgal concentrates by flocculation and their assessment as aquaculture feeds. Aquacultural Engineering 2006; 35:300–313.

Heasman M., Diemar J., O’Connor W., Sushames T., Foulkes L., Nell J.A. Development of extended shelf-life microalgae concentrate diets harvested by centrifugation for bivalve molluscs—a summary. Aquaculture Research 2000; 31(8 – 9):637– 59.
[69] Dassey A., Theegala C. Harvesting economics and strategies using centrifugation for cost effective separation of microalgae cells for biodiesel applications Bioresource Technology 2013; 128:241-245.

[70] Coward T., Lee J., Caldwell G. Harvesting microalgae by CTAB-aided foam flotation increases lipid recovery and improves fatty acid methyl ester characteristics Biomass and Bioenergy 2014; 67:354-362.

[71] Muñoz R., Guieysse B. Algal-bacterial processes for the treatment of hazardous contaminants: a review. Water Research 2006; 40(15):2799–815.

[72] Bruton T., Lyons H., Lerat Y., Stanley M., Rasmussen M.B. A review of the potential of marine algae as a source of biofuel in Ireland. Dublin: Sustainable Energy Ireland; 2009. p. 88.

[73] Uduman N., Qi Y., Danquah M.K., Forde G.M., Hoadley, A. Dewatering of microalgal cultures: a major bottleneck to algae-based fuels. Journal of Renewable and Sustainable Energy 2010; 2:012701–012715.

[74] Garg S., Wang L., Schenk P. Effective harvesting of low surface-hydrophobicity microalgae by froth flotation. Bioresource Technology 2014; 159:437–441.

[75] Nurra C., Torras C., Clavero E, Rios S., Rey M, Lorente E., Farriol X., Salvadó J. Biorefinery concept in a microalgae pilot plant. Culturing, dynamic filtration and steam explosion fractionation. Bioresource Technology 2014; 163:136-142

[76] Jacob-Lopes E, Teixeira Franco T. From oil refinery to microalgal biorefinery. Journal of CO₂ Utilization 2013; 2:1-7.

[77] Guldhe A., Singh B., Rawat I., Ramluckan K., Bux F. Efficacy of drying and cell disruption techniques on lipid recovery from microalgae for biodiesel production. Fuel 2014; 128(15):46-52.

[78] Goettel M., Eing C., Gusbeth C., Straessner R., Frey W. Pulsed electric field assisted extraction of intracellular valuables from microalgae. Algal Research 2013; 2(4): 401-408.

[79] Frey W., Gusbeth C., Schwartz T. Inactivation of Pseudomonas putida by pulsed electric field treatment: a study on the correlation of treatment parameters and inactivation efficiency in the short-pulse range. Journal of Membrane Biology 2013; 246(10): 769-781.

[80] Ranjan A., Patil C., Moholkar V.S. Mechanistic assessment of microalgal lipid extraction. Industrial & Engineering Chemistry Research 2010; 49(6):2979–2985.

[81] Ehimen E., Sun Z., Carrington G. Use of Ultrasound and Co-Solvents to Improve the In-Situ Transesterification of Microalgae Biomass. Procedia Environmental Sciences 2012; 15:47-55.
[82] Fu C-C., Hung T-C., Chen J-Y., Su C-H., Wu W-T. Hydrolysis of microalgae cell walls for production of reducing sugar and lipid extraction. Bioresource Technology 2010; 8750

[83] Sathish A., Sims R. Biodiesel from mixed culture algae via a wet lipid extraction procedure. Bioresource Technology 2012; 118:643–647.

[84] Richmond A. Handbook of microalgal culture: biotechnology and applied phycology. Blackwell Science Ltd; 2004.

[85] Belarbi H., Molina E., Chisti Y. A process for high and scaleable recovery of high purity eicosapentaenoic acid esters from microalgae and fish oil. Enzyme and Microbial Technology 2000; 26:516–29.

[86] Araya B., Gouveia L., Nobre B., Reis A., Chamy R., Poirrier P. Evaluation of the simultaneous production of lutein and lipids using a vertical alveolar panel bioreactor for three Chlorella species. Algal Research, In Press, Corrected Proof, Available online 4 July 2014

[87] Du Y., Schuur B., Samori C., Tagliavini E., Brilman DWF. Secondary amines as switchable solvents for lipid extraction from non-broken microalgae. Bioresource Technology 2013; 149: 253-260

[88] Amin Hejazi M., Wijffels R.H. Milking of microalgae. Trends in Biotechnology 2004; 22(4):189–94.

[89] Abrahamsson V., Rodriguez-Meizoso I., Turner C. Determination of carotenoids in microalgae using supercritical fluid extraction and chromatography. Journal of Chromatography A 2012; 1250:63-68.

[90] Solana M, Rizza C.S., Bertucco A. Exploiting microalgae as a source of essential fatty acids by supercritical fluid extraction of lipids: Comparison between Scenedesmus obliquus, Chlorella protothecoides and Nannochloropsis salina. The Journal of Supercritical Fluids 2014; 92:311-318.

[91] Hammann S., Tillmann U., Schröder M., Vetter W. Profiling the fatty acids from a strain of the microalgae Alexandrium tamarense by means of high-speed counter-current chromatography and gas chromatography coupled with mass spectrometry. Journal of Chromatography A 2013; 1312:93-103.

[92] Christenson L., Sims R. Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. Biotechnology Advances 2011; 29:686–702.

[93] Pulz O., Gross W. Valuable products from biotechnology of microalgae. Applied Microbiology and Biotechnology 2004; (65):635–48.

[94] Sheih I.C., Fang T., Wu TK., Lin PH. Anticancer and antioxidant activities of the peptide fraction from algae protein waste. Journal of Agricultural and Food Chemistry 2010; 58 (2):1202–1207.
[95] Becker W. Microalgae in human and animal nutrition. In: Richmond A, (ed.) Handbook of microalgal culture. Oxford: Blackwell Publishing; 2004. p312–51.

[96] Yen H.W., Brune D.E. Anaerobic co-digestion of algal sludge and waste paper to produce methane. Bioresource Technology 2007; 98:130-134.

[97] Miao X., Wu Q. Biodiesel production from heterotrophic microalgal oil. Bioresource Technology 2006; 97:841-846.

[98] Chae S.R., Hwang E.J., Shin H.S. Single cell protein production of *Euglena gracilis* and carbon dioxide fixation in an innovative photo-bioreactor. Bioresource Technology 2006; 97:322-329.

[99] Spolaore P., Cassan C.J., Duran E., Isambert A. Commercial applications of microalgae. Journal of Bioscience and Bioengineering 2006; 101:87-96.

[100] Muñoz, J., Mudge, S.M., Sandoval, A. Effects of ionic strength on the production of short chain volatile hydrocarbons by *Dunaliella salina* (Teodoresco) 2004; Chemosphere 54: 1267-1271.

[101] Dragone, G., Fernandes, B.D., Abreu, A.P., Vicente, A.A., Teixeira, J.A., 2011. Nutrient limitation as a strategy for increasing starch accumulation in microalgae. Applied Energy 2011; 88(10):3331–3335.

[102] Ho S-H., Huang S-W., Chen C-Y., Hasunuma T., Kondo A., Chang J-S. Bioethanol production using carbohydrate-rich microalgal biomass as feedstock. Bioresource Technology 2013; 135:191–198

[103] Ryckebosch E., Bruneel C., Muylaert K., Foubert I. Microalgae as an alternative source of omega-3 long chain polyunsaturated fatty acids. Lipid Technology 2012; 24(6):128–130.

[104] Ryckebosch E., Bruneel C., Termote-Verhalle R., Goiris K., Muylaert K., Foubert I. Nutritional evaluation of microalgae oils rich in omega-3 long chain polyunsaturated fatty acids as an alternative for fish oil. Food Chemistry 2014; 160:393–400.

[105] Yaakob Z., Ali E., Zainal A., Mohamad M., Takriff M.S. An overview: biomolecules from microalgae for animal feed and aquaculture. Journal of Biological Research-Thessaloniki 2014; 21:6.

[106] Harun R., Singh M., Forde G.M., Danquah M.K. Bioprocess engineering of microalgae to produce a variety of consumer products. Renewable & Sustainable Energy Reviews 2010; 14:1037–1047.

[107] Rodrigues D., Flores E., Barin J, Mercadante A, Jacob-Lopes E, Zepka Q.L. Production of carotenoids from microalgae cultivated using agroindustrial wastes. Food Research International, In Press, Corrected Proof, Available online 4 July 2014.
[108] Gerwick W.H., Tan L., Sitachitta N. Nitrogen-containing metabolites from marine cyanobacteria. In Cordell, G.A. (ed.) The Alkaloids. San Diego: Academic Press; 2001. p75-184

[109] Kenekar A., Deodhar M. Effect of Varying Physicochemical Parameters on the Productivity and Phycobiliprotein Content of Indigenous Isolate Geitlerinema sulphureum. Biotechnology 2013; 12(3):146-154.

[110] Sialve B., Bernet N., Bernard O. Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. Biotechnology Advances 2009; 27:409–416.