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Review

Microalgae for biotechnological applications: Cultivation, harvesting and biomass processing

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ABSTRACT

In either unicellular or multi-cellular form, microalgae are photosynthetic microorganisms, mainly known for being part of the human diet in several world regions. More recently, they have been in the spotlight of researchers, not only because of their nutritional value, but also due to their high value-added components. This work reviews five microalgae genera: *Dunaliella*, *Botryococcus*, *Chlamydomonas*, *Chlorella* and *Arthrospira*, considered among the most promising for commercial biotechnological applications. The analysis shows that, although the research paradigms are generally shared among species, parameterization changes of culture environment and stress conditions, several applications can be envisaged for the cultivated species, which is discussed in this work. Besides, several applications in which these microalgae are being widely used, or are intended to be used, are analyzed and discussed. The potential applications depend on the type of metabolites found in each microalgae species, which is discussed in this work, giving examples of application and describing methods for their cultivation, harvesting and biomass processing. Thus, in addition to being used in human diet supplementation, microalgae can be used as ingredients for animal feed, medicines, cosmetics pigments, biofuels, bioplastics and biostimulants.

1. Introduction

Microalgae are microorganisms, either in mono or multicellular form that comprise eukaryotic protists and prokaryotic cyanobacteria. While eukaryotic microalgae include both diatoms and green algae, the other group, so called prokaryotic microalgae comprises the cyanobacteria (Mobin and Alam, 2017). Microalgae can be found anywhere sunlight and water coincide, such as in soils, ice, lakes, rivers, hot springs and oceans (Zullaikah et al., 2019).

Various studies have shown that microalgae cultivation systems can absorb up to 200 times more CO_2 than trees (Melis, 2009). However, specific levels of carbon dioxide are required for different types of microalgae. For example, Herador (2016) reported that in general, a carbon dioxide concentration ranging from 0.038 to 10% is suitable for microalgae cultivation.

Several screening programs in different world locations have studied microalgae species to identify the most suitable strains. However, most research work has focused on a small number of fast growing microalgae species which accumulate substantial amounts of desirable components (Mobin and Alam, 2017). Among these, this review article focus on the following five typical genera *Dunaliella*, *Botryococcus*, *Chlamydomonas*, *Chlorella* and *Arthrospira*, considered most promising for commercial biotechnological purposes, reviewing their potential applications depending on the main characteristics and chemical composition, and giving some examples of cultivation, harvesting and biomass processing methods.

2. Brief presentation of the microalgae species

Microalgae are classified into cyanobacteria (blue-green), rhodophytes (red algae), chlorophytes (green algae) and chromophytes (all other algae) (Mobin and Alam, 2017).

Dunaliella, *Botryococcus*, *Chlamydomonas* and *Chlorella* are microalgae belonging to the phylum of chlorophytes that have different structural shapes. *Chlorella* has a spherical shape, as well as *Dunaliella*, however, this presents a change in its structural shape as the cells grow. *Chlamydomonas* and *Botryococcus* have oval shape, which depending on the species, may also present pyramidal shape. *Arthrospira* (*Spirulina*) are cyanobacteria with cylindrical trichome shape.

As shown in Table 1, *Botryococcus* can reach the highest amount of

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Table 1
Microalgae main characteristics and chemical composition (dry weight %).

Microalgae	Shape	Lipid content (dw %)	Protein conten (dw %)	Carbohydrate content (dw %)	Reference
Dunaliella	Changes with growth	25–75	50–80	10–25	Ahmed et al., 2017; Gomes, 2017
Botryococcus	Pyramidal or oval	25–75	3–10	17–21	Weiss et al., 2011; Alessandro and Antoniosi, 2016
Chlamydomonas	Oval	20–25	47–50	15–20	Shumbulo and Ki, 2018; Zullaikah et al., 2019
Chlorella	Spherical	14–56	10–58	10–17	Mata et al., 2016; Safi et al., 2014
Arthrospira platensis (Spirulina)	Cylindrical trichomes	7–23	57–65	20–30	Tandon and Jin, 2017; Mata et al., 2016

intracellular lipids (75% dw). On the other hand, *Arthrospira* shows the lowest lipid content (7–23% dw). Although some *Chlorella* species produce only 14% dw of lipids, there are species that can reach up to 56% dw. *Dunaliella* generally presents high protein content, reaching up to 80% dw, while *Chlamydomonas* can reach up 50% dw of protein content. *Arthrospira* species generally present high protein (57–65% dw). *Botryococcus* shows the lowest protein content (3–10% dw). Concerning carbohydrate rates, chlorophytes show similar rates ranging from 10 to 25% dw. Among the 5 genera studied, *Arthrospira* have the higher rates of carbohydrates ranging from 20 to 30% dw.

2.1. *Dunaliella*

Dunaliella is a chlorophyte microalga with complex life cycle of vegetative and sexual reproduction, which is able to accumulate active compounds such as chlorophyll, polyunsaturated fatty acids, lutein and zeaxanthin, considered high-efficient antioxidants and, in abundance, *β*-carotene. This microalga is unicellular and denucleated, has a fragile cell wall, and although the ideal growth salinity is approximately 22% saturation in the culture medium, it is able to grow in media with more than 30% sodium chloride saturation. This is because they balance the various external salinities by regulating the metabolism of osmolyte (Ye et al., 2008; Ben-Amotz and Avron, 1981).

In particular, *Dunaliella salina* and *Dunaliella bardawil* species are capable of accumulating greater amounts of carotenoids (8–14% of total dry weight) (Ye et al., 2008), and studies have shown that both species do not produce toxins and have no adverse effects when consumed by humans or animals (El-Baz et al., 2019; Regnier et al., 2015; Mokady et al., 1990; WEO/IPCS, 1993).

2.2. *Botryococcus*

Botryococcus belongs to the group of chlorophytes, forming pyramid-shaped colonies that can be found in temperate or tropical freshwater lakes and occasionally in estuaries and fjords around the world, where they are concentrated in large floating masses (Borowitzka, 2013). These microalgae accumulate in their extracellular matrix long chain hydrocarbons and ethereal lipids, similar to crude oil (Wake and Hillen, 1980; Brown et al., 1969), which reduce the density of colonies, leading to flotation on the water surface (Eroglu et al., 2011).

The most interesting and well-studied species is *Botryococcus braunii*, for its ability to produce hydrocarbons, especially triterpenes, in large quantities, commonly ranging between 30 and 40% of its dry weight (Metzger and Largeau, 2005). This species has a thick cell wall structure due to the accumulation of biomass from the previous cell divisions. Although this feature makes it difficult to extract cytoplasmic components, much of the useful hydrocarbon oil is extracellular (Wolf et al., 1985).

Botryococcus braunii is classified into four different biochemical races called: “race A” which contains large amounts of long-chain alkadienes and alkatrienes, oleic acid, complex mixtures of n-aldehydes and complex lipids (Volkman, 2014; Kawachi et al., 2012); “race B" that contains branched isoprenoid hydrocarbons (botryococcenes) and as minor compounds squalene is its methylated mono-, di-, tri- and tetramethylsqualene derivatives (Achitouv et al., 2004; Huang and Poulter, 1989; Metzger et al., 1985); “race L” contains one isoprenoid lycopadiene, two epoxides, a variety of lycopanerols which are tetraterpenoid ethers and high molecular weight polyaldehydes (Metzger et al., 2003); finally, “race S” is similar to race A, producing n-alkanes and epoxy alkanes (Kawachi et al., 2012).

Among the *Botryococcus braunii* races, “race B” is the one of main interesting species in the scientific field because of its high capacity to accumulate botriococenes (up to 86% of its dry weight) which are considered a promising renewable energy source for hydrocracking, and methylqualenes compounds easily converted to biofuels (Banerjee et al., 2002; Hillen et al., 1982; Brown et al., 1969).

2.3. Chlamydomonas

Chlamydomonas are unicellular green algae (Chlorophyta), formed by a cell wall, chloroplast and two flagella that they use to move around (Zullaikah et al., 2019). They can be found in soil, freshwater, oceans and even snow, on mountain tops. They are recognized as reference algae due to the huge amount of information that has been gathered from decades of studies involving their genome sequence, for providing an excellent microbial platform employed in the investigation of fundamental biological functions, and for having characteristics associated with plant and animal lineages (Blaby et al., 2014).

Even after millions of years of evolution, these genera of algae have retained its auxiliary components and photosynthetic system as its ancestors (Becker, 2013). Also, can be grown both in the presence and absence of light with acetate as the sole carbon source (Rolland et al., 2009). *Chlamydomonas* have flagella equivalent to mammalian flagella and cilia, which distinguish them from terrestrial plants (Pazour and Witman, 2009; Silflow and Lefebvre, 2001).

Over 500 different species of *Chlamydomonas* are catalogued, but the most widely used in scientific studies and research is *Chlamydomonas reinhardtii* which are haploid and can grow in simple medium of inorganic salts, using solar energy to perform photosynthesis (Zullaikah et al., 2019).

2.4. Chlorella

Chlorella can easily reproduce in the presence of sunlight, carbon dioxide, water and small amounts of nutrients, making their cultivation easy. They have a simple life cycle and superior plant-like metabolic pathways, being able to produce high amounts of protein, carotenoids, vitamins and minerals, and therefore are produced as source of healthy food (Silva et al., 2019).

Moreover, *Chlorella* accumulate high concentrations of carotenoids (astaxanthin, lutein, β-carotene, violaxanthin and zeaxanthin), antioxidants, vitamins, polysaccharides, proteins, peptides and fatty acids (Mata et al., 2010; Safi et al., 2014). Due to their high lipid content (ranging from 14 to 63% of dry weight), *Chlorella* is also considered a promising source for biofuel production, which can be grown without competing with human food crops (Caetano et al., 2020a; Frumento et al., 2013; Mata et al., 2016). After lipid transesterification for biodiesel, the residual biomass can be used to produce other biofuels such as methane, bio-oil and ethanol (Sakarika and Kornaros, 2019). Of the many species of *Chlorella*, the most commonly used in scientific studies and commercial production are *Chlorella vulgaris*, *Chlorella* sp. and *Chlorella pyrenoidosa*.

2.5. Arthrospira

Arthrospira commercially known as *Spirulina*, is a filamentous photosynthetic cyanobacteria recognized by the World Health Organization as one of the major superfoods due to their high protein content (ranging from 50 to 70% dry weight) and essential amino acids (Mitra and Mishra, 2017). It also contains high concentrations of B complex and E vitamins, minerals, bioactive compounds, antioxidants and carotenoids such as zeaxanthin, cryptoxanthin, C-phycocyanin, β-carotene and lutein (Ma et al., 2019). These microalgae can be easily grown in tropical and subtropical lakes. It adapts to extreme environments such as alkaline media, saline environments, high temperatures and light intensity. Also, it is relatively less susceptible to contamination than other microalgae species, making it the most favorable choice for large scale production. About 60% of all large-scale algal biomass produced is attributed to *Arthrospira* (Mitra and Mishra, 2017).

Arthrospira can be used for biofuels production, although with typical lipid content of 4–7% and 15–25% carbohydrates (Ljubic et al., 2018; Mata et al., 2016). This is because it accumulates up to 60–65% carbohydrates under phosphorus limitation, which through alcoholic fermentation can be converted into ethanol or butanol (Markou et al., 2013).

The two species of this microalgae considered most important due to their physicochemical characteristics, presented in scientific studies and research, are *Arthrospira maxima* and *Arthrospira platensis* (Soni et al., 2017).

3. Microalgae cultivation, harvesting and biomass processing

3.1. Cultivation

Microalgae are microorganisms that naturally grow in lakes, rivers and oceans, but in order to be applied industrially, these natural ecosystems are unsatisfactory due to the low biomass concentrations, which makes it difficult to harvest on a large scale. Microalgae grow fast in comparison to terrestrial plants, can reach high lipid content ($>$ 50%) and have other high value-added components, as already mentioned in the previous sections.

They are easily grown in medium containing inorganic nutrients such as nitrogen, phosphorus and CO_2 required by microalgae by approximately 180% of their dry weight (Chew et al., 2018; Singh and Dhar, 2011). Microalgae can also be grown in wastewater, using the nutrients available in the medium, while treating the water. Moreover, they have the capacity to remove heavy metals and other pollutants from industrial effluents (Cai et al., 2013).

Factors such as temperature, pH and light intensity in the culture medium, directly influence the growth and development of microalgal biomass. In addition, the presence of other microorganisms such as bacteria, fungi and viruses can affect the culture (Okoro et al., 2019). Temperature is an important factor as it alters the biochemical processes in the microalgae cell synthesis. Most species grow better at temperatures between 20 and 30 °C, except for thermophilic algae that tolerate temperatures up to 40 °C (Suparmaniam et al., 2019). Another important factor influencing microalgae growth is the medium pH, where the optimal pH range between 6 and 9 for most species to grow (Khan et al., 2018). Finally, light intensity, spectral composition and photoperiod are other important factors that directly affect the microalgae cells growth (Suparmaniam et al., 2019).

The culturing conditions directly influence the growth characteristics and cellular composition of microalgae, resulting in different biomass concentration and productivity, as well as variation in the content of lipids, carbohydrates, proteins and of other components (Chojnacka and Marquez-Rocha, 2004). Although different forms of cultivation and production have been developed in recent years, it is still necessary to find an effective and sustainable production and cultivation mechanism to reach the full potential of microalgae biomass, especially in large scale industrial applications (Anyanwu et al., 2018).

Dunaliella are strict photoautotrophs capable of absorbing CO_2 and HCO_3 for photosynthesis. Thus, the supply of inorganic carbon is important for the culture of this genus due to the low solubility of inorganic carbon presented in the high salinity medium in which this microalga grows. Moreover, it is very important that the medium contains sources of nitrogen (nitrate is the best source), phosphorus (which should have a concentration between 0.020 and 0.025 g/L to prevent growth inhibition), magnesium, calcium, chloride, sulfate, iron (in low concentrations) and trace elements such as Zn, Co, Cu, Mo and Mn. The Johnson's medium was optimized to meet all nutritional needs for *Dunaliella* cultivation (Table 2). In addition, cultivation should be done at temperature of 23 ± 2 °C with light intensity of approximately 200 $\mu Em^2 s^{-1}$ under a 12/12 h light/dark cycle (Ahmed et al., 2017; Oren, 2005; Johnson et al., 1968).

The Bold Basal Medium (BBM) is considered as a good standard cultivation medium for many classes of green freshwater algae (Gualtieri and Barsanti, 2006). *Botryococcus* and *Chlorella* grow well in

Table 2
Most common cultivation parameters for microalgae growth.

Microalgae	Cultivation[a]	Reference
Dunaliella	Johnson's medium; temperature of 23 ± 2 °C; light intensity of ≈ 200 μEm^2 s^{-1} under a 12/12 h light/dark cycle	Ahmed et al., 2017; Oren, 2005; Johnson et al., 1968
Botryococcus	Bold's Basal Medium (BBM); temperature of 25–27 °C; on a fluorescent light under a 15/9 h light/dark cycle	Dunker and Wilhelm, 2018; Velichkova et al., 2012; Weiss and Wilhelm, 2011
Chlamydomonas	Tris-acetate-phosphate (TAP) medium, pH 7; temperature of 25 ± 1 °C; on a continuous light 120 μEm^2 s^{-1}	Zullaikah et al., 2019; Shumbulo, 2018; Kong et al., 2010
Chlorella	Bold's Basal Medium (BBM); temperature of 22–30 °C; light intensity of 100–150 μEm^2 s^{-1} under a 16/8 h light/dark cycle	Safi et al., 2014; Frumento et al., 2013; Yamamoto et al., 2004
Arthrospira	Zarrouk's medium, pH 9; temperature of 30 ± 2 °C; light intensity of 30 μEm^2 s^{-1} under a 12/12 h light/dark cycle	Tandon and Jin, 2017; Mata et al., 2016; Madkour et al., 2012

[a] The most commonly used medium for the microalgae growth.

this medium under similar conditions of temperature and light. As shown in Table 2, *Botryococcus* grows better at temperatures between 25 and 27 °C on a fluorescent light under 15/9 h light/dark cycle (Dunker and Wilhelm, 2018; Velichkova et al., 2012; Weiss et al., 2011), whereas *Chlorella* grows better at temperatures between 22 and 30 °C with a light intensity of 100–150 μEm^{2}s^{-1} under a 16/8 h light/dark cycle (Safi et al., 2014; Frumento et al., 2013; Yamamoto et al., 2004).

The tris-acetate-phosphate (TAP) medium is a standard culture medium often used for *Chlamydomonas* (Table 2). Ammonium (NH_4^+) serves as a primary source of nitrogen and Tris buffer the pH and keeps it neutral. Furthermore, this medium contains a relatively low concentration of phosphate, which prevents the inhibition of biomass growth. *Chlamydomonas* grow better at a temperature of 25 °C, on a continuous light of 120 μEm^{2}s^{-1} (Zullaikah et al., 2019; Shumbulo and Ki, 2018; Kong et al., 2010).

The *Arthrospira* growth mainly depends on the availability of nutrients, temperature and light. The Zarrouk's medium is considered the standard medium for growing this microalgae (Madkour et al., 2012) that grows better at 30 °C, light intensity of 30 μEm^{2}s^{-1} and under 12/12 h light/dark cycle (Table 2).

3.2. Harvesting

Microalgae harvesting may include mechanical, chemical, biological or electrical methods, each of which with their own advantages and disadvantages. There is no single most suitable harvesting method for each microalgae genera; in general, the ideal is to use a method that preserves the biomass composition and does not hinder the recovery of the product of interest. The most common harvesting methods include filtration (Monte et al., 2018), centrifugation, flotation, flocculation (Caetano et al., 2020b), electroflocculation (Zenouzi et al., 2013), gravity sedimentation, electrolytic process, electrophoresis and magnetic separation (Safi et al., 2014). However, combining harvesting methods with a previous step of chemical or biological coagulation/flocculation thickening can increase process efficiency and reduce the operating costs (Grima et al., 2003).

Centrifugation is the most common process for microalgae harvesting as it is a fast and efficient method for cells recovery. It involves the application of centrifugal forces for separating microalgal biomass from the growth medium and, once separated, they are simply removed by draining off the excess water (Harun et al., 2010). Although considered one of the most efficient methods, with yields up to 98%, the main disadvantages are the high energy costs and the fact that the gravitational and shear forces during centrifugation process may damage the cell structure.

Filtration is also a common method for microalgae harvesting. It consists of recovering the solid phase (microalgae biomass) from the liquid phase (culture medium) through the meshes of a filter or membrane. Conventional filtration processes are vacuum filtration, pressure filtration, microfiltration, ultrafiltration, dead-end filtration and tangential flow filtration (Dragone et al., 2010). Ultrafiltration and microfiltration techniques (using smaller pore membranes) are used to recover biomass in smaller microalgae cultures (Petrusevski et al., 1995). The main costs of this process are associated with frequent replacement of filtration membranes due to clogging and energy consumption of the pumping system, but when compared to other harvesting methods, filtration is one of the most effective and economic competitive (Dragone et al., 2010). Membrane filtration is an efficient method to harvest *Dunaliella* and *Arthrospira* (Monte et al., 2018; Cuellar-Bermudez et al., 2019).

Flocculation can be used as a previous step to biomass recovery, as it consists of microalgae agglomeration, facilitating and increasing the efficiency of the subsequent processes (Caetano et al., 2020b). The repulsion charge between microalgae is neutralized or reduced by using flocculants such as aluminum sulfate salts ($Al_2(SO_4)_3$), iron chloride ($FeCl_3$) and iron sulfate ($Fe_2(SO_4)_3$), among others (Mata et al., 2016). Fan et al. (2017) used metal cations and medium pH 11 to harvest *Chlamydomonas reinhardtii*, reaching over 90% of *Chlorella vulgaris* biomass recovery by using chitosan and aluminum sulfate (Zhu et al., 2018). Some microalgae species may naturally flocculate (self-flocculation) from environmental stimuli, such as changes in the culture medium pH (Vandamme et al., 2013), and can naturally cluster by establishing a well-defined sedimentation rate. Other microalgae species can synthesize chemicals excreted in the outer cells producing a colloidal suspension that makes them difficult to settle. Besides, some species are mobile and do not settle naturally (Okoro et al., 2019).

Combining flocculation with a mechanical harvesting technique can improve biomass recovery at a reasonable cost (Caetano et al., 2020b; Duan and Gregory, 2003). Zenouzi et al. (2013) achieved 97.44% efficiency in harvesting *Dunaliella salina* using electroflocculation. By bioflocculation using *Aspergillus fumigatus*, Al-Hothaly et al. (2015) reached 98% *Botryococcus braunii* biomass recovery. Using moringa seeds as bioflocculant and pH 11, Maghfiroh et al. (2018) harvested *Chlorella* sp. biomass with a 98.44% of recovery efficiency.

Flotation is another simple method used to facilitate biomass harvesting (Chen et al., 2015). It uses air microbubbles dispersed in an aqueous medium and the microalgae cells are adsorbed and dragged to the surface of the aqueous medium to be removed (Brennan and Owende, 2010).

3.3. Biomass processing

Biomass processing may follow two routes, the first one for applications where microalgae must be applied dry, and the second one, where the application of methods to extract target components require the use of wet biomass.

3.3.1. Drying

Microalgae are cultivated in aqueous medium and, although harvesting results in higher biomass concentration, a drying step must be considered for several applications, such as food supplement (usually marketed as powders or pellets), or before lipid extraction (e.g. for biodiesel production). The drying process requires a lot of energy (e.g. to evaporate 1 kg of water, at least 800 kcal of energy is required) resulting in a final biomass concentration of at least 90% (Chen et al., 2015). Thus, the drying step can be an economic bottleneck of the entire process due to the high energy consumption.

The most common drying methods include freeze-drying, drum drying, spray drying and sun drying (Grima et al., 2003). However, the main drying method used for many microalgae genera (including the five genera focused in this review) is spray drying (Hosseinizand et al., 2018). Spray drying has advantages such as high versatility, the possibility to pack directly, the powder is produced without need of any grinding process and the ease of processing control, allowing the product quality to remain constant (uniform) during processing. However, its main disadvantage is the high capital and energy/operating costs (Chen et al., 2015).

3.3.2. Pre-treatment

Pre-treatment methods are used to increase the recovery of intracellular microalgae components, which may be carried out by chemical methods (e.g. acids, bases, supercritical fluids) (Lee et al., 2010; Crampon et al., 2013), physical methods (e.g. lyophilization, sonication, microwave, ultrasonography) (Adam et al., 2012; Biller et al., 2013), biological methods (enzyme or protein degradation) (Kita et al., 2010; Mercer and Armenta, 2011) and mechanical methods (e.g. high temperature autoclave, pressing, ball milling, electroporation, homogenization and osmotic shock) (Lee et al., 2012; McMillan et al., 2013).

Preservation of the chemical composition in microalgae biomass is an important factor for selecting the pre-treatment method, since the chemical components of biomass can be degraded by both the extraction process and the enzymatic activity present inside the microalgae cells (Chen et al., 2015). Also, the choice of the method to be used to break the cell wall and membrane depends on the cells morphology.

The pre-treatment methods presented in this section can be applied to *Dunaliella*, *Botryococcus*, *Chlamydomonas*, *Chlorella* and *Arthrospira*. The choice of which method to use depends on the desired product quality and recovery efficiency.

For example, high-pressure homogenization (HPH) is a common mechanical method used for large-scale cell disruption and components recovery, allowing high recovery of biological products. However, it is unsuitable for compounds such as lipids and proteins. This is because, there is a non-selective product release, with a high concentration of cellular debris, hindering the subsequent processes (extraction and purification). Additionally, it is a high energy consumption process (Norton and Sun, 2008; Balasundaram et al., 2009; Lee et al., 2013).

The application of electrical techniques such as pulsed electric field (PEF) and high voltage electric discharges (HVED) are promising and efficient methods for intracellular bio-suspensions extraction, and the PEF process is useful for lipid extraction allowing selective extraction of intracellular microalgae compounds (Lebovka et al., 2011; Gottel et al., 2011; Foltz, 2012). On the other hand, the HVED process achieves a more powerful mechanical disintegration of cell walls and can be applied more effectively to the extraction of higher molecular weight compounds, such as proteins (Liu et al., 2013).

3.3.3. Components extraction

Extraction is an operation in which one or more components in liquid or solid phase are transferred to another liquid phase by differences in component solubility. In liquid-liquid extraction it is used a solvent relatively immiscible with the solution, which is capable of removing the desired compound.

Techniques for extracting the desired components from microalgae biomass may be based or not on organic solvents and can be applied to the 5 microalgae genera studied in this review. The main factors that influence the selection of the most suitable extraction process for each of the desired components are the biochemical characteristics of microalgae cells, the intended final application of the extracted components, the adequacy of the solvent to be used, the expected extraction yield and time, the cost and ease of the process operation (Cuellar-Bermudez et al., 2014).

Organic solvent extraction is the most common technique for extracting microalgae components. For example, in solvent-based lipids extraction from wet biomass, it is necessary to increase the solvent polarity in order to reduce the barrier between non-polar solvents and intracellular lipids (Naghdi et al., 2016). Also, to recover proteins from microalgal biomass, it is necessary to remove interfering molecules such as lipids, carbohydrates, pigments and nucleic acids (Khanra et al., 2018). And, in carotenoid extraction, the biggest disadvantage is the maceration stage that requires a long process time (Parniakov et al., 2015).

Typical organic solvents applied to the extraction of intracellular microalgae compounds include methanol, chloroform, hexane, isopropanol, acetone and diethyl ether (Folch et al., 1957; Hara and Radin, 1987; Chen et al., 1984; Shao et al., 2015). The process yield depends on the operating parameters (e.g. temperature, pressure and nature of solvent).

Although extraction using organic solvents is the most common technique to recover microalgae biomolecules, the environmental and safety issues are the main limitations to the application of this method. Several research studies have been carried out to develop methods with solvent-free approaches such as microwave-assisted (Lee et al., 2010), ultrasound-assisted (Harun et al., 2010), hydrothermal liquefaction (Barreiro et al., 2013), Osmotic shock (Yoo et al., 2012; Prabakaran and Ravindran, 2011; Lee et al., 2010), enzymatic disruption (Demuez et al., 2015; Mercer and Armenta, 2011), oxidative stress (Bai et al., 2014), supercritical carbon dioxide (Halim et al., 2010), among others. The utilization of solvents which, in addition to being safer are more environmentally friendly, minimize the need to separate contaminants from the extracted product (Naghdi et al., 2016).

An alternative method for extracting microalgae components is the supercritical fluid extraction, which is a simpler, faster and more efficient than other conventional methods (Gouveia et al., 2007). Besides, it avoids the consumption of large quantities of organic solvents, and supercritical CO_2 has some unique characteristics as a solvent as it is non-toxic (Mendes et al., 2003).

4. Potential applications of microalgae

Probably one of the broadest applications of microalgae is in the food and feed industry, due to their high nutritional value and health benefits. For that purpose, they are normally commercialized in dry or wet forms (Raja et al., 2016). Other potential microalgae applications have also been studied, such as bioplastics, food colorants, cosmetics, bio-stimulants and biofuels, among others (Ramaraj et al., 2017).

Algal biomass can also be employed in industry (e.g. for medicines, cosmetics and nutraceutics), or for ecological applications (Lu et al., 2016; Borowitzka, 2013; Pignolet et al., 2013). For example, there are microalgal species used in environmental pollutants monitoring and bioremediation, playing an important role by providing a key tool useful for phytoremediation of both toxic metals and nanometals (Khatoon and Pal, 2015). *Dunaliella, Botryococcus, Chlamydomonas, Chlorella and Arthrospira* have several applications in industry, not only as a final product but also, as ingredients for other products formulation. The microalgae applications in different industry segments depend on the characteristics and composition of each microalgae biomass.

4.1. Food and feed industry

Several research studies have been conducted, showing that microalgae species such as those from the genus of *Chlorella* and *Spirulina* are suitable to be used in their pure forms (Herador, 2016; Raja et al., 2016), which explains their wide usage in the food industry, as for

Table 3
Potential applications of different microalgae species in food industry.

Microalgae	Component	Application	Reference
Chlorella zofingiensis	● Astaxanthin ● Provitamin A ● β-carotene ● Vitamin E ● Vitamin B12 ● Thiamin B1 ● Biotin ● Riboflavin B2 ● Niacin B3 ● Folic acid ● Vitamin B6 ● Inositol ● Phantothenic acid	● Dietary aid for leprosy patients ● Developing several trial food products (powdered green tea, soups, noodles, bread and rolls, cookies, ice cream, and soy sauce (	Barrow and Shahidi (2007); Mata et al. (2010); Liu et al. (2014); Belay et al. (1993); Belay et al. (1994)
Arthrospira platensis	● Protein (60–71% dw) ● Carbohydrate (13–16% dw)	● Vegan replacement ● Produce low cholesterol eggs ● Increase the number of lactic acid bacteria	Raja et al. (2008); Herador (2016); Becker (2007); Sajilata et al. (2008); Mobin and Alam (2017); Niccolai et al. (2019); Spolaore et al. (2006)
Dunaliella salina	● Protein (57% dw) ● Carbohydrate (32% dw) ● High levels of antioxidant ● Carotenoid β-content	● Human health dietary supplements (tablets, capsules, fortified nutritional blends) ● Animal feed ● Coloring margarine water-soluble ● Coloring beverages ● Prevent the intracellular oxidative stress	Raja et al. (2008); Raja et al., 2018; Hemaiswarya and Raja (2010); Capelli and Cysewski (2013); Spolaore et al. (2006); Mobin and Alam (2017)

example to enrich noodles, cookies, nutritional bars and juices (Chacon-Lee and Gonzalez-Marino, 2010; Desai and Sivakami, 2004).

Chlorella zofingiensis, *Arthrospira platensis* and *Dunaliella salina* are the species with most potential applications in the food industry due to its physical-chemical characteristics (Table 3).

Chlorella zofingiensis contains protein, lipids, carbohydrates, and a complex rich in vitamins, minerals and carotenoids, for this reason it has been applied as a powerful ingredient in several food products trials, such as powdered green tea, soups, noodles, bread and rolls, cookies, ice cream and soy sauce (Liu et al., 2014).

Due to high protein content (60–71% dw) and low carbohydrate content (13–16% dw), *Arthrospira platensis* has been applied in the vegan diet as a substitute for other protein and carbohydrates sources (Becker, 2007). In addition, this species has been applied to produce bioactive substances that promote lactic acid bacteria growth (Niccolai et al., 2019).

Dunaliella salina has been consumed as dietary supplements for human health in the form of pills, capsules, and fortified nutritional mixtures (Mokady et al., 1990). It is also applied in animal feed, due to its average contents of protein (57% dw) and carbohydrates (32% dw) (Raja et al., 2018). Also, it has been used as a natural food and in beverages, for producing carotenoids (9-cis-β-carotene), and in preventing intracellular oxidative stress due to its high antioxidant levels (Ye et al., 2008; Garcia-Gonzalez et al., 2005).

Gill and Valivety (1997) states that algal based feed affects the animal's physiology as well as it improves its immune response and fertility. These authors have shown that the combination of 5–10% of algal biomass, mixed with animal feed, can be used safely as partial replacement for conventional proteins, while higher ratio may present adverse effects. These authors showed that recent research studies on the quality of proteins with microalgal sources have a comparable or superior functionality (either as foam, emulsifier, surfactant, solubilizer or gelling agent) equal or even greater than other commercial protein sources. As an instance, they pointed to *Chlorella* and showed that it can be digested easily up to 5% in the form of paste (Harari et al., 2013). This market includes aquaculture industries, as microalgae are known to be the most appropriate source for fish feed in all stages of marine bivalve mollusks, larva stages of some gastropods, some fish larvae, penaeid shrimp and zooplankton (Raja et al., 2018). Microalgae can provide an important source of essential fatty acid to fish. Omega-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the most famous compounds in fish oil for human consumption (Martinez-Porchas et al., 2014). This point is important because DHA and EPA are not synthesized by fish and the only resource to enrich them in fish is by accumulating them in proper algal paste and store them while fish feed (Martinez-Porchas et al., 2014).

Chini-Zitelli et al. (1999) reviewed the procedure required to provide the algae paste, initiated from cultivation, and then processed and condensed in the form of pastes (concentrated microalgae cells dispersed in liquid media) or freeze-dried cubes. Algae paste is produced by batch cultivation of microalgae such as *Chlorella vulgaris* and *Arthrospira platensis* and accompanied by further dewatering via centrifugation. Finally, food grade preservatives are added to the dried paste to extend their expiration date. The resulting wet paste is then stored at 0–4 °C. It should be mentioned that the second form, the freeze-dried cubes, provides a good baseline to store under refrigeration for long term transportation (Hemaiswarya and Raja, 2010). These frozen cubic feedstocks can then be added directly to the water tank to feed the targeted marine animals (Raja et al., 2018).

4.2. Medicine

According to the World Health Organization, microalgae are promising sources of bioactive compounds (e.g. polysaccharides, carotenoids, vitamins and sterols) with various health benefits, such as anti-atherosclerostic or anti-cancer properties benefits, and for reducing cholesterol (Borowitzka, 2013; Geppert et al., 2006; Lordan et al., 2011; Raja et al., 2018). Microalgae contain long-chain polyunsaturated fatty acids (LC-PUFAs), such as eicosapentaenoic acid (EPA), and can function as a natural antibacterial medicine against both gram-positive and gram-negative bacteria (Desbois et al., 2009; Benkendorff et al., 2005). This behavior has benefits as immune-stimulants both for human and marine health (Shah et al., 2018; Falaise et al., 2016). Also, have shown that algal extracts, processed or crude, can be used in replacement or as supplement in shrimp or fish feed against bacterial infections (Yaakob et al., 2014; Dang et al., 2011).

Table 4 lists the potential medical applications for *Dunaliella salina*, *Chlorella vulgaris* and *Arthrospira platensis* that are common species applied in medicine.

Dunaliella salina has been studied for both pharmaceutical and nutraceutical applications, such as anti-Parkinson's disease, detoxifying heavy metals, supplement for protecting eye cells, antioxidant, neutralizing free radicals, anti-hypertensives, broncholitic and analgesics (Leya et al., 2009; Bhosale and Bernstein, 2008; Spolaore et al., 2006;

Table 4
Potential medical applications of different microalgae species.

Microalgae	Component	Application	Reference
Dunaliella salina	• β-carotene • α-carotene • Cryptoxanthin • Zeaxanthin • Lutein • Glutathione	• Anticancer activity • AntiParkinson's disease • Detoxify the metals • Protect eye cells • Antioxidant activity • Neutralizing the free radicals • Antihypertensive • Broncholytic • Analgesic drugs	Leya et al. (2009); Bhosale and Bernstein (2008); Spolaore et al. (2006); Villar et al. (1992)
Chlorella zofingiensis	• Astaxanthin • β-carotene • Glucan	• Increase hemoglobin concentrations • Lower blood sugar levels • Act as hypocholesterolemic and hepatoprotective agents during malnutrition • Ethionine intoxication • Stimulates immune response • Lowering lipids in the blood	Barrow and Shahidi (2007); Varfolomeev and Wasserman (2011); Paniagua-Michel et al. (2015)
Arthrospira platensis	• Phycocyanin (colorant) • γ -Linolenic acid (GLA) • Vitamin B1 • Leucine • Isoleucine valine • Provitamin A • Vitamin B_{12} • Vitamin K • β-carotene • Essential polyunsaturated fatty acids (PUFA)	• Anticancer • Antioxidant • Anti-inflammatory • Prevent viral infections • Increase the production of plaminogen-activating factor • Cholesterol reduction • Immune system enhancement • Anti-tumor • Prevent schizophrenia • Prevent dermatitis, prevent multiple sclerosis • Prevent diabetes Prevent rheumatoid arthritis	Spolaore et al. (2006); Belay et al. (1993); Patil et al. (2008); Paniagua-Michel et al. (2015); Becker (2007); Sajilata et al. (2008); Mobin and Alam (2017); Richmond (2004)

Villar et al., 1992). This species has the capability to store essential carotenoids such as α and β-carotene, cryptoxanthin, zeaxanthin and lutein, up to 14% dw with antioxidant and anticancer properties and the antioxidant glutathione (Ye et al., 2008; Metting Jr, 1996; Bruno et al., 2016). Also, it can be consumed in powder or tablet forms, as supplement for protein, amino-acid, minerals, vitamin C, B_{12} and fucose (Martinez-Hernandez et al., 2018).

As previously reported *Chlorella zofingiensis* is a species rich in β-carotene. Besides, the polysaccharide compounds found in this species it has been related to antitumor effects (Lu et al., 2016). Since it is rich in β-1,3-glucan, which is an active immune-stimulator, it can be used as a free radical scavenger, while it can control blood lipids (Spolaore et al., 2006). This microalga is a source of astaxanthin, a compound belonging to xanthophyll type, with antioxidant and anticancer properties (Ranga Rao et al., 2014; Capelli and Cysewski, 2013). Astaxanthin can be used as nutritional supplement with human health benefits such as anti-inflammatory and anticancer agent and it is widely used in pharmacology to cure cardiovascular diseases (Regnier et al., 2015; Chacon-Lee and Gonzalez-Marino, 2010). Also, as shown by Walker et al. (2005) it has positive effects to prevent diabetes type 2 and neurodegenerative disorders.

Besides its applications in food industry, *Arthrospira platensis* has also been pointed for medical usages (Ramaraj et al., 2017). Containing both phycobiliproteins (an active free radical scavenger) and high amount of γ-linolenic acid (GLA) and essential polyunsaturated fatty acids (PUFA), it is reported to enhance the immune system and prevent tumor development (Richmond, 2004). Also, Spolaore et al. (2006) refer that *Arthrospira platensis* has the capability to alleviate hyperlipidemia and suppress hypertension, while it can protect body against renal failure. In addition, this species of microalgae is capable of producing vitamins and β-carotene, and therefore it is applied in medicine as antioxidant, anti-inflammatory, to prevent viral infections, increase the production of plaminogen-activating factor, immune system enhancement, to prevent diseases as schizophrenia, dermatitis, multiple sclerosis, diabetes and rheumatoid arthritis (Belay et al., 1993; Patil et al., 2008; Paniagua-Michel et al., 2015; Sajilata et al., 2008; Richmond, 2004).

4.3. Pigments

Although there are more than 400 known carotenoids, only very few are used commercially. These carotenoids include β-carotene, astaxanthin lutein, zeaxanthin, lycopene and bixin (Del Campo et al., 2000; Vilchez et al., 1997). Some carotenoids can act as provitamin A and thus, can be converted in the body to vitamin A (Garcia-Gonzalez et al., 2005). Carotenoids have medical applications and can be used as ingredient for nutritional supplements and, due to its color, it can be used as colorant for both human food and animal feed (Gouveia and Empis, 2003).

The green halophilic flagellate *Dunaliella salina* is the most suitable microalgae for the mass cultivation of β-carotene, with a content of about 14% of its dry weight (Ye et al., 2008). It can be cultivated outdoors in open ponds as it is known to be a robust specimen to extreme environmental conditions such as hypersaline water, nitrogen deprivation, together with high levels of solar irradiation (Metting Jr, 1996).

Chlorella pigments have also been studied to be used as coloring agent and for taste and flavor-adjustments in healthy food industry (Gouveia and Empis, 2003; Yamaguchi, 1997).

The US Food and Drug Administration (FDA) has regulations to employ the phycocyanin pigment obtained from *Spirulina platensis* as color additive in gum, toffee and other food items (Julianti et al., 2019; Spolaore et al., 2006).

Due to the fact that they produce β-carotene, *Dunaliella salina* are applied as a natural colorant for aquaculture salmon feedstock and *Chlorella zofingiensis* are applied as pigmenter for salmon (Table 5). *Arthrospira platensis* produce phycocyanin and are being used as pigments in chewing gum, ice sherbets, popsicles, candies, soft drinks,

Table 5
Potential pigments applications of different microalgae species.

Microalgae	Component	Application	Reference
Dunaliella salina	• β-carotene	• Natural colorant for aquaculture feedstock (Salmon)	Spolaore et al. (2006)
Chlorella zofingiensis	• β-carotene • α-carotene	• Pigmenter for salmon	Ramaraj et al. (2017)
Arthrospira platensis	• Phycocyanin (Phycobili- protein)	• Chewing gum, ice sherberts, popsicles, candies, soft drinks, dairy products and wasabi	Becker (2007)

dairy products and wasabi (Table 5).

4.4. Cosmetics

Cosmeceuticals, cosmetic products with bioactive ingredients, some of which obtained from microalgae are in high demand in the market. The use of extracts from microalgae species, in particular of *Arthrospira* and *Chlorella*, is well established in the cosmetics market and can be found in anti-aging creams, refreshing and regenerating products, emollients, peelers and hair and sun protection products. Microalgae extracts provide numerous properties for the final application of products, such as preventing stretch marks in the skin, exerting a skin hardening effect, stimulating collagen synthesis and repairing the signs of premature skin aging. Additionally, the high value extracts from microalgae have a potential not only for anti-aging skin care products but also as antimicrobial, thereby extending the already wide range of applications into the cosmeceutical area (Spolaore et al., 2006; Mourelle et al., 2017).

Table 6 show the potential cosmetics applications by *Arthrospira platensis*, *Chlorella zofingiensis* and *Chlorella vulgaris*, and *Dunaliella salina*.

Microalgae compounds obtained from *Arthrospira platensis* can be used in skin care products such as anti-aging cream, refreshing or regenerative care products, as well as anti-irritation and sun protection creams (Kim et al., 2008). Studies stated the positive effect of *Spirulina*, containing hyaluronic acid derivatives, for moisturizing skin layers (Morais et al., 2014). Protulines, a protein-rich extract from *Arthrospira*, helps combat early skin aging and can also prevent wrinkle formation (Raja et al., 2018; Spolaore et al., 2006).

Chlorella zofingiensis are being used in skin care cosmetics since it has hydrating, moisturizing and anti-aging benefits, as UV-screening agent, and for sunscreen and hair care. *Chlorella vulgaris* has the ability for smoothing and confer elasticity to skin (Sathasivam et al., 2019; Ariede et al., 2017; Safafar et al., 2015; Goiris et al., 2012). Besides, there are analysis supporting the effective influence of *Chlorella vulgaris* on stimulating collagen synthesis, which plays an important role in skin regeneration and wrinkle reduction (Stolz and Obermayer, 2005).

Dunaliella salina extracts containing phenols (e.g., gallic, caffeic, salycilic, p-coumaric, and ferulic acid) are used as stimulants to energy metabolism of skin cells, promoting skin regeneration (Varfolomeev and Wasserman, 2011). Carotenoids have also applications in cosmetics (Ariede et al., 2017). As previously mentioned in Section 4.3, β-carotene from *Dunaliella salina* is employed as natural colorant in foods (Garcia-Gonzalez et al., 2005). However, the cosmetic industry is also interested in its natural colorant (Hosseini et al., 2013). For instance, there are companies investing on red pigments obtained from red microalgae as colorants for cosmetic products including lipstick, eye shadow and face make-up (Ariede et al., 2017).

4.5. Biofuel

Extensive research was conducted on microalgae as a new source for biodiesel production because of their high lipids content together with high biomass productivity, having the capacity to partly replace the demand for vegetable oils obtained from terrestrial crops (Herador, 2016). Considering biodiesel production, research studies have shown that the microalgae oil yield is larger than that obtained per hectare from palm oil, soybean or rapeseed (Martinez-Porchas et al., 2014; Deng et al., 2009). Alson, microalgae is considered to be a promising feedstock for third generation biofuels, being 10–20 times more productive than any other biofuel crop (Hannon et al., 2010; Mata et al., 2010; Ndimba et al., 2013). Besides, they do not compete with human food crops and can be cultivated in non-arable land. Also, they can be cultivated using saline water and wastewater (Brennan and Owende, 2010; Christenson and Sims, 2011). Therefore, microalgae showing a great potential to solve problems such as cultivation of large farming

Table 6
Potential cosmetics applications of different microalgae species.

Microalgae	Component	Application	Reference
Arthrospira platensis	• Protein-rich	• Early skin aging • Exerts a tightening effect • Prevents strain formation • Anti-aging skin • Skin care • Hair care • Sun protection	Raja et al., 2018; Morais et al. (2014); Kim et al. (2008); Spolaore et al. (2006)
Chlorella Zofingiensis	• Colorful pigments	• Skin care • Hair care • Hydrating skin • Moisturizing • Antiaging benefits • UV-screening agent • Sunscreen	Spolaore et al. (2006)
Chlorella vulgaris	• Protein • Phenols (e.g., gallic, caffeic, salycilic, p-coumaric, and ferulic acid)	• stimulating collagen synthesis • supporting tissue regeneration reducing skin wrinkle • regenerating creams for face and body lotions	Sathasivam et al. (2019); Safafar et al. (2015); Goiris et al. (2012)
Dunaliella Salina	• Glycerol, Phenols (e.g., gallic, caffeic, salycilic, p-coumaric, and ferulic acid)	• Moisturize skin • Smoothen skin	Ramaraj et al. (2017); Safafar et al. (2015); Goiris et al. (2012)

land and use of fresh water resources.

There are several challenges to use microalgae for biofuel purposes. According to Herador (2016), the main challenge is to reach a high biomass production, providing the required environmental conditions suitable for microalgae growth. Also, as described before, bioreactors and open-pond systems are widely employed for the production of microalgal biomass commercially. Herador (2016) compared the possible environmental conditions to find the most practical option. In one side, in closed PBRs, it is possible to better control microalgae growth and maintain the monoculture of lipid-rich algal strains. On the other side in open raceways, these are real shortcomings, as the control of other species growth is much more difficult. However, open-pond systems are preferred for producing microalgae biomass for biodiesel due to their easier implementation and lower investment and operating costs. The other challenge is harvesting of microalgae from large volumes. Preparation of the harvested strains to make them ready for lipid extraction has been discussed in several research studies (as presented in section 3.3.3). While there are different techniques to harvest microalgae biomass most of them are not appropriate for the complete harvesting pipeline, thus requiring a combination of different techniques for better economic performance (Mallick et al., 2016). Another challenge is the management of the effluent resulting from the harvesting of biomass from the culture medium (Brennan and Owende, 2010; Dismukes et al., 2008).

Botryococcus and *Chlorella* are suitable microalgae for biodiesel production due to their ability to accumulate high lipid content under stress conditions (Caetano et al., 2020a; Frumento et al., 2013; Martins et al., 2016). In particular, *Botryococcus braunii* are able to reach a higher lipid content of 65%, containing 9.95%, 79.61% and 10.54% of respectively saturated, monounsaturated and polyunsaturated fatty acids, which makes it suitable for biodiesel production (Tasic et al., 2016).

Dunaliella can also be used for biofuels production, for example *Dunaniella tertiolecta* that can generate up to 25.8% of bio-oil yield by hydrothermal liquefaction of microalgal biomass residues (Shuping et al., 2010) and by methylation of different fatty acids, such as linolenic and palmitic acids (Tang et al., 2011). Nayeong et al. (2012) showed that *Duanliella salina* is also suitable for biogas production by anaerobic digestion due to its high biomass productivity, low cost for pre-treatments and easy processing.

Although *Arthrospira platensis* is known for its very low lipid content (Martins et al., 2016; Baunillo et al., 2012), under nutrients limitation it accumulates carbohydrates that can reach 60% - 65% of dry biomass (Markou et al., 2013). Baunillo et al. (2012) reported for *Arthrospira platensis* the largest lipid content of 20% achieved in growth under phosphorus and nitrogen deprivation, and obtained 42% of crude biodiesel yield, with 55% of fatty acids methyl ester (FAME) content that consisted mainly of C16: 0 and C18: 2, similar to that of conventional biodiesel (Luque et al., 2008). In another study, Markou et al. (2013) cultivated *Arthrospira platensis* in medium with phosphorus limitation for using the resulting carbohydrate-enriched biomass as substrate for the bioethanol production by fermentation, reaching the highest bioethanol yield of approximately 16 g of ethanol per gram of biomass.

4.6. Bioplastics

The world market for bioplastics is growing every year by 8–10%, showing an increase from $1bn in 2007 to $10bn by 2020 and fast-growing microalgae can be the source for biopolymers (Herador, 2016). Bioplastics are obtained through conversion of algal biomass via fermentation, plasticization, blending and compatibilization processing (Wang et al., 2014). Unlike soy or other feedstocks for bioplastics, the fermentation process is identified to be reliable and simple, making algal biomass a viable alternative for oil-based polymers. Plasticization includes a stage in which non-volatile organic molecules (such as glycerol, sorbitol, saccharose, urea, triethylene glycol, or polyethylene glycol) are added to the extracts to increase flexibility and durability. Another important stage is blending, where compatible polymers (polyethylene or vinyl alcohol) are added to the process. Finally, compatibilization is the stage in which the interfacial properties of the blended polymers are modified to stabilize the bioplastic. Bioplastics are then molded or extruded with both heat and pressure to be prepared for the intended application.

Different types of bioplastics can be produced, such as polyhydroxyalkanoates (PHAs), poly-lactic acid (PLA), starch plastics, cellulose plastics and protein plastics. PHAs are considered the most promising substitute for petroleum plastics because they have hydrophobicity such as polyethylene and polypropylene (Lambert and Wagner, 2017). Due to substantial biodegradability, excellent barrier properties and low toxicity, PLA is also considered a promising material for the production of bioplastics (Zhang et al., 2019; Lambert and Wagner, 2017). Another common polysaccharide that has been applied as a bioplastic is starch, which is composed of homoglucans and glucopyranose for the synthesis of amylose and amylopectin, biomolecules with three-dimensional structures and plastic properties (Zhang et al., 2019). In the same way that vegetable proteins have been widely used as raw material in the bioplastics production, proteins produced by microalgae such as *Chlorella* sp. and *Spirulina* sp. are considered as an alternative raw material for the production of bio-based plastics (Zeller et al., 2013).

A life cycle assessment for comparing petrochemical polymers with biodegradable biopolymers, concluded that despite results were generally unfavorable for biopolymers mainly due to the current immature technologies for their production, there is still a lot of room for optimization and improvements in process efficiencies (Yates and Barlow, 2013). Thus, it is expected that the environmental profile of biopolymers will continue to improve in the future.

In recent years, several studies have been published involving the synthesis of bioplastics from microalgae, such as from *Chlorella* (Das et al., 2018; Zeller et al., 2013), *Spirulina* sp. (Zhang et al., 2020; Zeller et al., 2013) and *Chlamydomonas* sp. (Mathiot et al., 2019).

Many reports indicate that *Chlorella* sp. can be exploited for the PLA production due to its high carbohydrates content (more than 60% dw) (Aikawa et al., 2012). Cyanobacteria such as *Spirulina* sp. use sunlight to convert CO_2 and produce up to 10% dw of PHAs (Haase et al., 2012). Mathiot et al. (2019) showed that *Chlamydomonas reinhardtii* can be used to produce a considerable amount of starch bioplastics with glycerol in a twin-screw extruder. Regarding the use of proteins as raw material for bioplastic production, Verdugo et al. (2014) showed that *Botryococcus braunii* with a high protein content, processed by acid electrospinning, form fibers of 200 nm in diameter. Also, *Chlorella sorokinana* rich in proteins and polysaccharide adjunct, increased resistance to water vapor and improved the mechanical properties of bioplastics (Gomez-Heincke et al., 2017).

Bioplastics obtained from microalgae are certified to be used in various applications, including packaging, food transportation, gardening and medical hygiene (Wang et al., 2014; Rajendran et al., 2012).

4.7. Bio-stimulants

Recent environmental toxicology studies have shown the negative effects of chemical fertilizers in soil, plants and environment. In this regard, attention to organic farming have increased significantly in recent years (Uysal et al., 2015). Irregular use of phosphorus and nitrogen during cultivation of agricultural crops resulted in several environmental problems and reduction of crop yields, while application of synthetic fertilizer is associated with the increase in soil erosion (Dineshkumar et al., 2018). Although the cost of chemical fertilizers is increasing continuously, these chemicals are widely used and are responsible for environmental problems (Dineshkumar et al., 2018).

Microalgal biostimulants contain macro and micronutrients, phytohormones, carotenoids, amino acids, antifungal substances, growth regulators, polyamines, natural enzymes, carbohydrates, proteins and vitamins required for enhancing plant growth, being able to fix nitrogen, solubilize phosphate and promote microorganisms demanded for plants growth (Shaaban, 2001; Ronga et al., 2019). Biostimulants when applied in low doses to crops or soil, are able to regulate and improve the physiological processes of the plants. They act on the plant physiology in different ways: improving crop growth, nutrient absorption, tolerance to abiotic stresses, and increase the quality, productivity and shelf life of the harvested products (Ronga et al., 2019). Thus, the utilization of bio-stimulants in an eco-friendlier and resource-efficient way for replacing synthetic fertilizers, contributing to more sustainable agricultural practices (Sutton et al., 2013; Bloemberg et al., 2000).

Phytohormones found in microalgae extracts, including gibberellins, auxins, cytokinins, anscisic acid and ethylene, are known to influence plant growth and development (Ronga et al., 2019). Plaza et al. (2018) studied the effects of foliar spraying with *Scenedesmus* sp. and *Arthrospira platensis* htdrolysates on Petunia x hybrid plant. In this experiment *Scenedesmus* sp. showed higher concentration of cytokinins, gibberellins, auxins, salicylic acid and abscisic acid compared with *Arthrospira platensis*, which are phytohormones that accelerated plant development. Results showed that foliar applications of *Scenedesmus* sp. accelerated plant development in terms of higher root growth rates, leaf and shoot development, and earliness of flowering, while *Artrhospira platensis* hydrolysates enhaced the root dry matter, number of flowers per plant, the water content and improved the plant nutrient status.

Mahmoud (2001) evaluated the effects of using dry Chlorella vulgaris as soil additive on the nutrient status and root growth of maize plants. Results showed significant increase in nutrient taken up by roots and in roots volume, chlorophyll formation and olant height.

Abd El-Barky et al. (2009) shwed that water extract of *Spirulina maxima* and *Chlorella ellipsoida* can improve wheat tolerance to salinity, and enhanced the antioxidant capacity and protein content of the whole grains produced by treating plants with microalgal extracts.

Coppens et al. (2015) evaluated the application of microalgal biomass in tomato cultivars, showing that though the fruit quality improved through an increase in sugar and carotenoid content, a lower tomato yield was obtained. The analysis showed that tomatoes grown on microalgal-bacterial flocs contained 70% more carotenoids compared to the inorganic fertilizer treatment and 44% more than in the organic fertilizer treatment.

4.8. Wastewater treatment, CO_2 mitigation and bioremediation

Research studies proposed the use of some microalgae species for wastewater treatment, by removing nutrients from wastewater for their growth and improving the efficiency of treatment processes (Cai et al., 2013). Also, good results were reported when using microalgae for bioremediation of industrial wastes (Wang et al., 2010; Mata et al., 2014), including toxic gases (Chiu et al., 2011), heavy metals, petroleum contaminants, dyes (Cai et al., 2013; Doshi et al., 2007; Kong et al., 2010) and aquaculture effluents (Martinez-Porchas et al., 2014). As an instance, *Chlorella vulgaris* is reported to remove 72% of nitrogen and 28% for phosphorus in average as intakes from domestic wastewater (Rizwan et al., 2018).

Other solution is the symbiotic co-culturing of microalgae and bacteria, for which Contreras-Angulo et al. (2019) reported higher biomass productivity, while performing the treatment of Nitrogen-deficient wastewater from the agroindustry that is a significant problem posing difficulties to its biological treatment. These authors showed that symbiotic co-culturing can be effectively performed on N-deficient media, enhancing microalgae colony size and lipid content for biofuels.

Microalgae have higher CO_2 fixation rates when compared to terrestrial plants. Therefore, the capture of CO_2 and its conversion to carbohydrates and lipids in microalgal cells is a promising method for sequestration of CO_2 from gas emissions of combustion engines (Chiu et al., 2011).

Chlorella strains from hot springs can tolerate CO_2 concentrations up to 40% (*v*/v) (Rizwan et al., 2018). Chiu et al. (2011) studied the production of microalgal biomass and on-site bioremediation of carbon dioxide, nitrogen oxide and sulfur dioxide from flue gas using *Chlorella* sp. cultures, reaching an average of 60% CO_2 removal, 70% NO removal and 50% SO_2 removal from flue gas. This behavior may contribute significantly to reduce the amount of greenhouse gas.

5. Conclusion

Microalgae species, in both form of mono-cellular or multi-cellular, have been studied for several years for various applications. These microorganisms do not only can be cultured naturally in open environments such as ponds using natural resources, but also, they can be grown under specific conditions for commercial purposes. In recent decades, investigations have revealed that they can be used as a valuable resource in various areas; medical, animal feedstock, cosmetic purposes, and even biofuel production. This article focused on five typical microalgae genera considered among the most promising for commercial biotechnological purposes, reviewing their potential applications, cultivation, harvesting and biomass processing methods. Examples of applications for the high-value components and extracts from these five microalgae are presented, in particular for the production of medical supplements (vitamins and nutrients), pigments (natural edible colors) and cosmeceuticals.

Credit author statement

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Declaration of Competing Interest

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References

Abd El-Baky, H.H., El-Baz, F.K., El Baroty, G.S., 2009. Enhancing antioxidant availability in wheat grains from plants grown under seawater stress in response to microalgae extract treatments. J. Sci. Food Agric. 90, 299–303.

Achitouv, E., Metzger, P., Rager, M.-N., Largeau, C., 2004. C31–C34 methylated squalenes from a Bolivian strain of *Botryococcus braunii*. Phytochemistry. 65, 3159–3165.

Adam, F., Abert-Vian, M., Peltier, G., Chemat, F., 2012. "Solvent-free" ultrasoundassisted extraction of lipids from fresh microalgae cells: a green, clean and scalable process. Bioresour. Technol. 114, 457–465.

Ahmed, R.A., He, M., Aftab, R.A., Zheng, S., Nagi, M., Bakri, R., Wang, C., 2017. Bioenergy application of *Dunaliella salina* SA134 grown at various salinity levels for lipid production. Sci. Rep. 7, 8118.

Aikawa, S., Izumi, Y., Matsuda, F., Hasunuma, T., Chang, J., Kondo, A., 2012. Synergistic enhancement of glycogen production inArthrospira platensis by optimization of light intensity and nitratesupply. Bioresour Technol 108, 211–215.

Alessandro, E.B.D., Antoniosi, N.R., 2016. Concepts and studies on lipid and pigments of microalgae: a review. Renew. Sust. Energ. Rev. 58, 832–841.

Al-Hothaly, K.A., Adetutu, E.M., Taha, M., Fabbri, D., Lorenzetti, C., Conti, R., May, B.H., Shar, S.S., Bayoumi, R.A., Ball, A.S., 2015. Bio-harvesting and pyrolisis of the microalgae *Botryococcus braunii*. Bioresour. Technol. 191, 117–123.

Anyanwu, R., Rodriguez, C., Durrant, A., Olabi, A.-G., 2018. Microalgae cultivation technologies. In: Hashmi, S. (Ed.), Reference Module in Materials Science and Materials Engineering. Elsevier B.V, pp. 1–23.

Ariede, M.B., Candido, T.M., Jacome, A.L.M., 2017. Cosmetic attributes of algae - a review. Algal Res. 25, 483–487.

Bai, X., Naghdi, F.G., Ye, L., Lant, P., Pratt, S., 2014. Enhanced lipid extraction from algae using free nitrous acid pretreatment. Bioresour. Technol. 159, 36–40.

Balasundaram, B., Harrison, S., Bracewell, D.G., 2009. Advances in product release strategies and impact on bioprocess design. Trends Biotechnol. 27, 477–485.

Banerjee, A., Sharma, R., Chisti, Y., Banerjee, U.C., 2002. *Botryococcus braunii*: a renewable source of hydrocarbons and other chemicals. Crit. Rev. Biotechnol. 22, 245–279.

Barreiro, D.L., Prins, W., Ronsse, F., Brilman, W., 2013. Hydrothermal liquefaction (HTL) of microalgae for biofuel production: state of the art review and future prospects. Biomass Bioenergy 53, 113–127.

Barrow, C., Shahidi, F., 2007. Marine Nutraceuticals and Functional Foods, First edition. (Boca Raton).

Baunillo, K.E., Tan, R.S., Barros, H.R., Luque, R., 2012. Investigations on microalgal oil production from Arthrospira platensis: towards more sustainable biodiesel production. RSC Adv. 2 (30), 11267–11272.

Becker, E.W., 2007. Microalgae as a source of protein. Biotechnol. Adv. 25 (2), 207–210.

Becker, B., 2013. Snow ball earth and the split of Streptophyta and Chlorophyta. Trends Plant Sci. 18, 180–183.

Belay, A., Ota, Y., Miyakawa, K., Shimamatsu, H., 1993. Current knowledge on potential health benefits of *Spirulina*. J. Appl. Phycol. 5, 235–241.

Belay, A., Ota, Y., Miyakawa, K., Shimamatsu, H., 1994. Production of high quality *Spirulina* at earthrise farms. In: Phang, S.M., Lee, K., Borowitzka, M.A., Whitton, B. (Eds.), Algal Biotechnology in the Asia-Pacific Region. University of Kuala Lumpur, pp. 92–102.

Ben-Amotz, A., Avron, M., 1981. Glycerol and β-carotene metabolism in the halotolerant alga *Dunaliella*: a model system for biosolar energy conversion. Trends Biochem. Sci. 6, 297–299.

Benkendorff, K., Davis, A.R., Rogers, C.N., Bremner, J.B., 2005. Free fatty acids and sterols in the benthic spawn of aquatic molluscs, and their associated antimicrobial properties. J. Exp. Mar. Biol. Ecol. 316, 29–44.

Bhosale, P., Bernstein, P.S., 2008. Microbial xanthophylls. Appl. Microbiol. Biotechnol. 68, 445–455.

Biller, P., Friedman, C., Ross, A.B., 2013. Hydrothermal microwave processing of microalgae as a pre-treatment and extraction technique for bio-fuels and bioproducts. Bioresour. Technol. 136, 188–195.

Blaby, I.K., Blaby-Haas, C.E., Tourasse, N., Hom, E.F.Y., Lopez, D., Aksoy, M., Grossman, A., Umen, J., Dutcher, S., Porter, M., King, S., Witman, G.B., Stanke, M., Harris, E.H., Goodstein, D., Grimwood, J., Schmutz, J., Vallon, O., Merchant, S.S., Prochnik, S., 2014. The *Chlamydomonas* genome project: a decade on. Trends Plant Sci. 29 (10), 672–680.

Bloemberg, G.V., Wijfijes, A.H.M., Lamers, G.E.M., Stuurman, N., Lugtenberg, B.J.J., 2000. Simulataneous imaging of *Pesudomonas flourescens* WCS 3655 populations expressing three different autofluorescent proteins in rizosphere: new perspective for studying microbial communities. Mol. Plant Mic. Int. 13 (11), 1170–1176.

Borowitzka, M.A., 2013. High-value products from microalgae – their development and commercialization. J. Appl. Phycol. 25, 743–756.

Brennan, L., Owende, P., 2010. Biofuels from microalgae – a review of technologies for production, processing, and extractions of biofuels and co-products. Renew. Sust. Energ. Rev. 14 (2), 557–577.

Brown, A.C., Knights, B.A., Conway, E., 1969. Hydrocarbon content and its relationship to physiological state in the green alga *Botryococcus braunii*. Phytochemistry 8, 543–547.

Bruno, M., Koschmieder, J., Wuest, F., Schaub, P., Fehling-Kaschek, M., Timmer, J., Beyer, P., Al-Babili, S., 2016. Enzymatic study on AtCCD4 and AtCCD7 and their potential to form acyclic regulatory metabolites. J. Exp. Bot. 67 (21), 5993–6003.

Caetano, N., Melo, A.R., Gorgich, M., Branco-Vieira, M., Martins, A.A., Mata, T.M., 2020a. Influence of cultivation conditions on the bioenergy potential and bio-compounds of *Chlorella vulgaris*. Energy Rep. 6 (S1), 378–384.

Caetano, N.S., Martins, A.A., Gorgich, M., Gutiérrez, D.M., Ribeiro, T.J., Ribeiro, T.J., Mata, T.M., 2020b. Flocculation of *Arthrospira maxima* for improved harvesting. Energy Rep. 6 (S1), 423–428.

Cai, T., Park, S.Y., Li, Y.B., 2013. Nutrient recovery from wastewater streams by microalgae: status and prospects. Renew. Sust. Energ. Rev. 19, 360–369.

Capelli, B., Cysewski, G., 2013. Natural astaxanthin: The World's Best Kept Health Secret. Cyanotech Corporation, Kailua-Kona, HI (ISBN 978-0-9792353-0-6).

Chacon-Lee, T.L., Gonzalez-Marino, G.E., 2010. Microalgae for healthy foods possibilities and challenges. Compr. Rev. Food Sci. Food Saf. 9, 655–675.

Chen, I.S., Shen, C.S.J., Sheppard, A.J., 1984. Comparison of methylene chloride and chloroform for the extraction of fats from food products. J. Am. Oil Chem. Soc. 58, 599–601.

Chen, C.-L., Chang, J.-S., Lee, D.-J., 2015. Dewatering and drying methods for microalgae. Dry. Technol. 33 (4), 443–454.

Chew, K.W., Chia, S.R., Show, P.L., Yap, Y.J., Ling, T.C., Chang, J.-S., 2018. Effects of water culture medium, cultivation systems and growth modes for microalgae cultivation: a review. J. Taiwan Inst. Chem. E. 91, 332–344.

Chini-Zitelli, G., Lavista, F., Bastianini, A., Rodolfi, L., Vicenzini, M., Tredici, M.R., 1999. Production of eicosapentanoic acid by *Nannochloropsis* sp. cultures in outdoor tubular photobioreactors. Prog. Ind. Microbiol. 35, 299–312.

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

Chojnacka, K., Marquez-Rocha, F.-J., 2004. Kinetic and stoichiometric relationships of the energy and carbon metabolism in the culture of microalgae. Biotechnology 3 (1), 21–34.

Christenson, L., Sims, R., 2011. Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. Biotechnol. Adv. 29, 686–702.

Contreras-Angulo, J.R., Mata, T.M., Cuellar-Bermudez, S.P., Caetano, N.S., Chandra, R., Garcia-Perez, J.S., Muylaert, K., Parra-Saldivar, R., 2019. Symbiotic co-culture of *Scenedesmus* sp. and *Azospirillum brasilense* on N-deficient media with biomass. Produc. Biofuels Sustain. 11 (3), 707.

Coppens, J., Grunert, O., Van Den Hende, S., Vanhoutte, I., Boon, N., Haesaert, G., De Gelder, L., 2015. The use of microalgae as a high-value organic slow-release fertilizer results in tomatoes with increased carotenoid and sugar levels. J. Appl. Phycol. 28 (4), 2367–2377.

Crampon, C., Mouahid, A., Toudji, S.-A.A., Lepine, O., Badens, E., 2013. Influence of pretreatment on supercritical CO_2 extraction from *Nannochloropsis oculata*. J. Supercrit. Fluids 79, 337–344.

Cuellar-Bermudez, S.P., Aguilar-Hernandez, I., Cardenas-Chavez, D.L., Ornelas-Soto, N., Romero-Ogawa, M.A., Parra-Saldivar, R., 2014. Extraction and purification of high-value metabolites from microalgae: essential lipids, astaxanthin and phycobiliproteins. Microb. Biotechnol. 8, 190–209.

Cuellar-Bermudez, S.P., Kilimtzidi, E., Devaere, J., Goiris, K., Gonzalez-Fernandez, C., Wattiez, R., Muylaert, K., 2019. Harversting of *Arthrospira platensis* with helicoidal and straight trichomes using filtration and centrifugation. J. Contrib. https://doi.org/10.6084/m9.figshare.8228855.v2.

Dang, V.T., Li, Y., Speck, P., Benkendorff, K., 2011. Effects of micro and macroalgal diet supplementations on growth and immunity of green lip abalone, *Haliotis laevigata*. Aquaculture 320, 91–98.

Das, S.K., Sathish, A., Stanley, J., 2018. Production of biodiesel and bioplastics from *Chlorella Pyrenoidosa*. Mater. Today-Proc. 5 (8), 16774–16781.

Del Campo, J.A., Moreno, J., Rodriguez, H., Vargas, M.A., Rivas, J., Guerrero, M.G., 2000. Carotenoid content of chlorophycean microalgae: factors determining lutein accumulation in *Muriellopsis* sp. (Chlorophyta). J. Biotechnol. 76, 51–59.

Demuez, M., Mahdy, A., Tomas-Pejo, E., Gonzalez-Fernandez, C., Ballesteros, M., 2015. Enzymatic cell disruption of microalgae biomass in biorefinery processes. Biotechnol. Bioeng. 112, 1955–1966.

Deng, X., Li, Y., Fei, X., 2009. Microalgae: a promising feedstock for biodiesel. Afr J Microbiol Res 3, 1008–1014.

Desai, K., Sivakami, S., 2004. *Spirulina* the wonder food of the 21st century. APBN 8, 1298–1302.

Desbois, A.P., Mearns-Spragg, A., Smith, V.J., 2009. A fatty acid from the diatom *Phaeodactylum tricornutum* is antibacterial against diverse bacteria including multi-resistant *Staphylococcus aureus* (MRSA). Mar. Biotechnol. 11, 45–52.

Dineshkumar, R., Kumaravel, R., Gopalsamy, J., Sikder, M.N.A., Sampathkumar, P., 2018. Microalgae as bio-fertilizers for rice growth and seed yield productivity. Waste Biomass Valorization 9 (5), 793–800.

Dismukes, G.C., Carrieri, D., Bennette, N., Ananyev, G.M., Posewitz, M.C., 2008. Aquatic phototrophs: efficient alternatives to land-based crops for biofuels. Curr. Opin. Biotechnol. 19, 235–240.

Doshi, H., Ray, A., Kothari, I.L., 2007. Bioremediation potential of live and dead *Spirulina*: spectroscopic, kinetics and SEM studies. Biotechnol. Bioeng. 96, 1051–1063.
Dragone, G., Fernandes, B.D., Vicente, A., Teixeira, J.A., 2010. Third generation biofuels from microalgae. Appl. Microbiol. Microb. Biotechnol. 2, 1355–1366.
Duan, J., Gregory, J., 2003. Coagulation by hydrolysing metal salts. Adv. Colloid Interf. Sci. 100, 475–502.
Dunker, S., Wilhelm, C., 2018. Cell wall structure of coccoid green algae as an important trade-off between biotic interference mechanisms and multidimensional cell growth. Front. Microbiol. 9, 719. https://doi.org/10.3389/fmicb.2018.00719.
El-Baz, F.K., Aly, H.F., Salama, A.A.A., 2019. Toxicity assessment of the green *Dunaliella* salina microalgae. Toxicol. Rep. 6, 850–861.
Eroglu, E., Okada, S., Melis, A., 2011. Hydrocarbon productivities in different *Botryococcus* strains: comparative methods in product quantification. J. Appl. Phycol. 23, 763–775.
Falaise, C., Francois, C., Travers, M.-A., Morga, B., Haure, J., Tremblay, R., Turcotte, F., Pasetto, P., Gastineau, R., Hardivillier, Y., Leignel, V., Mouget, J.L., 2016. Antimicrobial compounds from eukaryotic microalgae against human pathogens and diseases in aquaculture. Mar. Drugs 14, 159. https://doi.org/10.3390/md14090159.
Fan, J., Zheng, L., Bai, Y., Saroussi, S., Grossman, A.A., 2017. Flocculation of *Chamydomonas reinhardtii* with different phenotypic traits by metal cation and high pH. Plant. Biotechnol. https://doi.org/10.3389/fpls.2017.01997.
Folch, J., Lees, M., SloaneStanley, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497–509.
Foltz, G., 2012. Algae Lysis With Pulsed Electric Fields. California Polytechnic State Universityhttps://doi.org/10.15368/theses.2012.45. (San Luis Obispo Follow).
Frumento, D., Casazza, A.A., Al Arni, S., Converti, A., 2013. Cultivation of *Chlorella vulgaris* in tubular photobioreactors: a lipid source for biodiesel production. Biochem. Eng. J. 81, 120–125.
Garcia-Gonzalez, M., Moreno, J., Manzano, J.C., Florencio, F.J., Guerrero, M.G., 2005. Production of *Dunaliella salina* biomass rich in 9-cis-β-carotene and lutein in a closed tubular photobioreactor. J. Biotechnol. 115, 81–90.
Geppert, J., Kraft, V., Demmelmair, H., Koletzko, B., 2006. Microalgal docosahexaenoic acid decreases plasma triacylglycerol in normolipidaemic vegetarians: a randomised trial. Br. J. Nutr. 95, 779–786.
Gill, I., Valivety, R., 1997. Polyunsaturated fatty acids, part 1: occurrence, biological activities and applications. Trends Biotechnol. 15, 401–409.
Goiris, K., Muylaert, K., Fraeye, I., Foubert, I., De Brabanter, J., De Cooman, L., 2012. Antioxidant potential of microalgae in relation to their phenolic and carotenoid content. J. Appl. Phycol. 24 (6), 1477–1486.
Gomes, D.I.C., 2017. Extraction and characterization of microalgae proteins from the extremophile *Dunaliella*. Técn. Lisboa 1–10.
Gomez-Heincke, D., Martínez, I., Stading, M., Gallegos, C., Partal, P., 2017. Improvement of mechanical and water absorption properties of plant protein based bioplastics. Food Hydrocoll. 73, 21–29. https://doi.org/10.1016/j.foodhyd.2017.06.022.
Gottel, M., Eing, C., Gusbeth, C., Frey, W., 2011. Pulsed electric field treatment of microalgae for cell ingredients extraction. In: 1st European Congress of Applied Biotechnology together with 29th DECHEMA's Biotechnology Annual Meeting.
Gouveia, L., Empis, J., 2003. Relative stabilities of microalgal carotenoids in microalgal extracts, biomass and fish feed: effect of storage conditions. Innov. Food Sci. Emerg. Technol. 4, 227–233.
Gouveia, L., Batista, A.P., Miranda, A., Empis, J., Raymundo, A., 2007. *Chlorella vulgaris* biomass used as colouring source in traditional butter cookies. Innov. Food Sci. Emerg. Tech. 4, 433–436.
Grima, E.M., Belarbi, E.H., Fernandez, F.G.A., Medina, A.R., Chisti, Y., 2003. Recovery of microalgal biomass and metabolites: process options and economics. Biotechnol. Adv. 20, 491–515.
Gualtieri, P., Barsanti, L., 2006. Algae : Anatomy, Biochemistry, and Biotechnology. Taylor & Francis, Boca Raton.
Haase, S.M., Huchzermeyer, B., Rath, T., 2012. PHB accumulation in *Nostoc muscorum* under different carbon stress situations. J. Appl. Phycol. 24 (2), 157–162. https://doi.org/10.1007/s10811-011-9663-6.
Halim, R., Gladman, B., Danquah, M.K., Webley, P.A., 2010. Oil extraction from microalgae for biodiesel production. Bioresour. Technol. 102, 178–185.
Hannon, M., Gimpel, J., Tran, M., Rasala, B., Mayfield, S., 2010. Biofuels from algae: challenges and potential. Biofuels 1, 763–784.
Hara, A., Radin, N.S., 1987. Lipid extraction of tissues with a low-toxicity solvent. Anal. Biochem. 90, 420–426.
Harari, A., Abecassis, R., Relevi, N., Levi, Z., Ben-Amotz, A., Kamari, Y., Harats, D., Shaish, A., 2013. Prevention of athersosclerosis progression by 9-cis-β-carotene rich alga *Dunaliella* in apoE-deficient mice. Biomed. Res. Int. https://doi.org/10.1155/2013/16951.
Harun, R., Singh, M., Forde, G.M., Danquah, M.K., 2010. Bioprocess engineering of microalgae to produce a variety of consumer products. Renew. Sust. Energ. Rev. 14 (3), 1037–1047.
Hemaiswarya, S., Raja, R., 2010. Microalgae and immune potential a chapter in Dietary Components and Immune Function edited by Ronald R. Watson, Sherma Zibadi, Victor R Preedy. Humana Press / Springer, USA, pp. 515–527.
Herador, M., 2016. The Microalgae/Biomass Industry in Japan. An Assessment of Cooperation and Business Potential with European Companies. EU-Japan Crntre for Industrial Cooperation.
Hillen, L.W., Pollard, G., Wake, L.V., White, N., 1982. Hydrocracking of the oils of *Botryococcus braunii* to transport fuels. Biotechnol. Bioeng. 24, 193–205.
Hosseini, S.M., Khosravi-Darani, K., Mozafari, M.R., 2013. Nutritional and medical applications of *Spirulina* microalgae. Mini Rev. Med. Chem. 13, 1231–1237.
Hosseinizand, H., Sokhansanj, S., Lim, C.J., 2018. Studying the drying mechanism of microalgae *Chlorella vulgaris* and the optimum drying temperature to preserve quality characteristics. Dry. Technol. 36 (9), 1049–1060.
Huang, Z., Poulter, C.D., 1989. Tetramethylsqualene, a triterpene from *Botryococcus braunii* var. *showa*. Phytochemistry 28, 1467–1470.
Johnson, M.K., Johnson, E.J., Mac Elroy, R.D., Speer, H.L., Bruff, B.S., 1968. Effects of salts on the halophilic alga *Dunaliella viridis*. J. Bacteriol. 95, 1461–1468.
Julianti, E., Susanti Singgih, M., Neti Mulyani, L., 2019. Optimization of extraction method and characterization of phycocyanin pigment from *Spirulina platensis*. J. Mathematic. Fundament. Sci. 51 (2), 168–176.
Kawachi, M., Tanoi, T., Demura, M., Kaya, K., Watanabe, M.M., 2012. Relationship between hydrocarbons and molecular phylogeny of *Botryococcus braunii*. Algal Res. 1 (2), 114–119.
Khan, M.I., Shin, J.H., Kim, J.D., 2018. The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. Microb. Cell. Factor. 17, 1–21.
Khanra, S., Mondal, M., Halder, G., Tiwari, O.N., Gayen, K., Bhowmick, T.K., 2018. Downstream processing of microalgae for pigments, protein and carbohydrate in industrial application: a review. Food Bioprod. Process. 110, 60–84.
Khatoon, N., Pal, R., 2015. Microalgae in biotechnological application: A commercial approach. In: Bahadur, B., Venkat, Rajam M., Sahijram, L., Krishnamurthy, K. (Eds.), Plant Biol. Biotechnol. Springer, New Delhi.
Kim, S.K., Ravichandran, Y.D., Khan, S.B., Kim, Y.T., 2008. Prospective of the cosmeceuticals derived from marine organisms. Biotechnol. Bioprocess Eng. 13, 511–523.
Kita, K., Okada, S., Sekino, H., Imou, K., Yokoyama, S., Amano, T., 2010. Thermal pretreatment of wet microalgae harvest for efficient hydrocarbon recovery. Appl. Energ. 87, 2420–2423.
Kong, Q.-X., Li, L., Martinez, B., Chen, P., Ruan, R., 2010. Culture of microalgae *Chlamydomonas reinhardii* in wastewater for biomass feedstock production. Appl. Biochem. Biotechnol. 160, 9–18.
Lambert, S., Wagner, M., 2017. Environmental performance of bio-based and biodegradable plastics: the road ahead. Chem. Soc. Rev. 46 (22), 6855–6871. https://doi.org/10.1039/c7cs00149e.
Lebovka, N., Vorobiev, E., Chemat, F., 2011. Enhancing Extraction Processes in the Food Industry. Series: Contemporary Food Engineering. CRC Press, Taylor & Francis LLC, pp. 592.
Lee, J.-Y., Yoo, C., Jun, S.-Y., Ahn, C.-Y., Oh, H.-M., 2010. Comparison of several methods for effective lipid extraction from microalgae. Bioresour. Technol. 101, 75–77.
Lee, A.K., Lewis, D.M., Ashman, P.J., 2012. Disruption of microalgal cells for the extraction of lipids for biofuels: processes and specific energy requirements. Biomass Bioenergy 46, 89–101.
Lee, A.K., Lewis, D.M., Ashman, P.J., 2013. Force and energy requirement for microalgal cell disruption: an atomic force microscope evaluation. Bioresour. Technol. 128, 199–206.
Leya, T., Rahn, A., Lutz, C., Remias, D., 2009. Response of arctic snow and permafrost algae to high light and nitrogen stress by changes in pigment composition and applied aspects for biotechnology. FEMS Microbiol. Ecol. 67, 432–443.
Liu, D., Lebovka, N.I., Vorobiev, E., 2013. Impact of electric pulse treatment on selective extraction of intracellular compounds from *Saccharomyces cerevisiae* yeasts. Food Bioprocess. Technol. 6 (2), 576–584.
Liu, J., Sun, Z., Gerken, H., Liu, Z., Jiang, Y., Chen, F., 2014. *Chlorella zofingiensis* as an alternative microalgal producer of astaxanthin: biology and industrial potential. Mar. Drugs 12 (6), 3487–3515.
Ljubic, A., Safafar, H., Holdt, S.L., Jacobsen, C., 2018. Biomass composition of *Arthrospira* platensis during cultivation on industrial process water and harvesting. J. Appl. Phycol. 30 (2), 943–954.
Lordan, S., Paul, R.R., Stanton, C., 2011. Marine bioactives as functional food ingredients: potential to reduce the incidence of chronic diseases. Mar. Drugs 9 (6), 1056–1100.
Lu, L., Georg, P., Wei, D., 2016. Extracellular metabolites from industrial microalgae and their biotechnological potential. Mar. Drugs 14 (10), 191.
Luque, R., Herrero-Davila, L., Campelo, J.M., Clark, J.H., Hidalgo, M., Luna, D., Marinas, J.M., Romero, A.A., 2008. Biofuels: a technological perspective. Energy Environ. Sci. 1, 542–564.
Ma, Z., Ahmed, F., Yuan, B., Zhang, W., 2019. Fresh living *Arthrospira* as dietary supplements: current status and challenges. Trends Food Sci. Technol. 88 (2019), 439–444.
Madkour, F.F., Kamil, A.E.-W., Nasr, H.S., 2012. Production and nutritive value of *Spirulina platensis* in reduced cost media. Egypt. J. Aquat. Res. 38 (1), 51–57.
Maghfiroh, W., Erdawati Saefurahman, G., Hidayatulloh, S., Kawaroe, M., 2018. Harversting Effectiveness of *Chlorella* sp. Biomass Using Different Flocculation Treatments of *Moringa oleifera* extract and pH Conditions (ICB2018). https://doi.org/10.1088/1755-1315/209/1/012014.
Mahmoud, M.S., 2001. Nutritional status and growth of maize plants as affected by green microalgae as soil additives. J. Biol. Sci. 1 (6), 475–479.
Mallick, N., Bagchi, S.K., Koley, S., Singh, A.K., 2016. Progress and challenges in microalgal biodiesel production. Front. Microbiol. 7, 1019.
Markou, G., Angelidaki, I., Nerantzis, E., Georgakakis, D., 2013. Bioethanol production by carbohydrate-enriched biomass of *Arthrospira* (*Spirulina*) *platensis*. Energy 6 (8), 3937–3950.
Martinez-Hernandez, G.B., Castillejo, N., Carrion-Monteagudo, M.M., Artes, F., Artes-Hernández, F., 2018. Nutritional and bioactive compounds of commercialized algae powders used as food supplements. Food Sci. Technol. Int. 24 (2), 172–182.
Martinez-Porchas, M., Martinez-Cordova, L.R., Lopez-Eliasb, J.A., Porchas-Cornejoc, M.A., 2014. Bioremediation of aquaculture effluents. Microb. Biodegrad. Bioremed. 24, 539–553.
Martins, A.A., Mata, T.M., Oliveira, O., Oliveira, S., Mendes, A.M., Caetano, N.S., 2016. Sustainability evaluation of biodiesel from *Arthrospira platensis* and *Chlorella vulgaris* under mixotrophic conditions and salinity stress. Chem. Eng. Trans. 49, 571–576.

Mata, T.M., Martins, A.A., Caetano, N.S., 2010. Microalgae for biodiesel production and other applications: a review. Renew. Sustain. Energ. Rev. 14, 217–232.

Mata, T.M., Mendes, A.M., Caetano, N.S., Martins, A.A., 2014. Sustainability and economic evaluation of microalgae grown in brewery wastewater. Bioresour. Technol. 168, 151–158.

Mata, T.M., Martins, A.A., Oliveira, O., Oliveira, S., Mendes, A., Caetano, N.S., 2016. Lipid content and productivity of *Arthrospira platensis* and *Chlorella vulgaris* under mixotrophic conditions and salinity stress. Chem. Eng. Trans. 49, 187–192.

Mathiot, C., Ponge, P., Gallard, B., Sassi, J.F., Delrue, F., Le Moigne, N., 2019. Microalgae starch-based bioplastics: screening of ten strains and plasticization of unfractionated microalgae by extrusion. Carbohydr. Polym. 208, 142–151. https://doi.org/10.1016/j.carbpol.2018.12.057.

McMillan, J.R., Watson, I.A., Ali, M., Jaafar, W., 2013. Evaluation and comparison of algal cell disruption methods: microwave, waterbath, blender, ultrasonic and laser treatment. Appl. Energ. 103, 128–134.

Melis, A., 2009. Solar energy conversion efficiencies in photosynthesis: minimizing the chlorophyll antennae to maximize efficiency. Plant Sci. 177, 272–280.

Mendes, R.L., Nobre, B., Cardoso, M., Pereira, A., Palavra, A., 2003. Supercritical carbon dioxide extraction of compounds with pharmaceutical importance from microalgae. Inorg. Chim. Acta 356, 328–334.

Mercer, P., Armenta, R.E., 2011. Developments in oil extraction from microalgae. Eur. J. Lipid Sci. Technol. 113, 539–547.

Metting Jr., F.B., 1996. Biodiversity and application of microalgae. J. Ind. Microbiol. 17, 477–489.

Metzger, P., Largeau, C., 2005. *Botryococcus braunii*: a rich source for hydrocarbons and related ether lipids. Appl. Microbiol. Biotechnol. 66 (5), 486–496.

Metzger, P., Casadevall, E., Pouet, M.J., Pouet, Y., 1985. Structures of some botryococcenes – branched hydrocarbons from the B race of the green alga *Botryococcus braunii*. Phytochemistry 24, 2995–3002.

Metzger, P., Rager, M.N., Sellier, N., Largeau, C., 2003. Lycopanerols I–L, four new tetraterpenoid ethers from *Botryococcus braunii*. J. Nat. Prod. 66, 772–778.

Mitra, M., Mishra, S., 2017. Multiproduct biorefinery from *Asthrospira* spp. towards zero waste: current status and future trends. Bioresour. Technol. 291, 121928. https://doi.org/10.1016/j.biortech.2019.121928.

Mobin, S., Alam, F., 2017. Some promising microalgal species for commercial applications: a review. Energ. Proc. 110, 510–517.

Mokady, S., Abramovici, A., Cogan, U., 1990. The safety evaluation of *Dunaliella bardawil* as a potential food supplement. Food Chem. Toxicol. 27 (4), 221–226.

Monte, J., Sá, M., Galinha, C.F., Costa, L., Hoekstra, H., Brazinha, C., Crespo, J.G., 2018. Harvesting of *Dunaliella salina* by membrane filtration at pilot scale. Sep. Purif. Technol. 190, 252–260.

Morais, M.G., Vaz, B.S., Morais, E.G., Costa, J.A.V., 2014. Biological effects of *Spirulina* (*Arthrospira*) biopolymers and biomass in the development of nanostructured scaffolds. Biomed. Res. Int. 762705. https://doi.org/10.1155/2014/762705.

Mourelle, M.L., Gómez, C.P., Legido, J.L., 2017. The potential use of marine microalgae and cyanobacteria in cosmetics and thalassotherapy. Cosmetics 4 (4), 46. https://doi.org/10.3390/cosmetics4040046.

Naghdi, F.S., González, L.M., Chan, W., Schenk, P.M., 2016. Progress on lipid extraction from wet algal biomass for biodiesel production. Microb. Biotechnol. 9 (6), 718–726.

Nayeong, J., Kim, D., An, J., Kim, T., Gim, G.H., Kang, C.M., Kim, D.J., Kim, S.W., Chang, I.S., 2012. *Dunaliella salina* as a microalgal biomass for biogas production. Korean J. Microbiol. Biotechnol. 4 (3), 282–285.

Ndimba, B.K., Ndimba, R.J., Johnson, T.S., Waditee-Sirisattha, R., Baba, M., Sirisattha, S., Shiraiwa, Y., Agrawal, G.K., Rakwal, R., 2013. Biofuels as a sustainable energy source: an update of the applications of proteomics in bioenergy crops and algae. J. Proteome 93, 234–244.

Niccolai, A., Shannon, E., Abu-Ghannam, N., Biondo, N., Rodolfi, L., Tredici, M.R., 2019. Lactic acid fermentation of *Arthrospira platensis* (*Spirulina*) biomass for probiotic-based products. J. Appl. Phycol. 31, 1077–1083.

Norton, T., Sun, D.-W., 2008. Recent advances in the use of high pressure as an effective processing technique in the food industry. Food Bioprocess Technol. 1, 2–34.

Okoro, V., Azimov, U., Munoz, J., Hernandez, H.H., Phan, A.N., 2019. Microalgae cultivation and haversting: growth performance and use of flocculants – a review. Renew. Sust. Energ. Rev. 115, 109364. https://doi.org/10.1016/j.rser.2019.109364.

Oren, A., 2005. A century of *Dunaliella research*: 1905–2005. In: Adaptation to Life at High Salt Concentrations in Archaea, Bacteria, and Eukarya. Springer, pp. 491–502.

Paniagua-Michel, J.J., Morales-Guerrero, E., Soto, J.O., 2015. Microalgal biotechnology: biofuels and bioproducts. In: Kim, S.-K. (Ed.), Springer Handbook of Marine Biotechnology. Springer, Berlin Heidelberg, pp. 1355–1370.

Parniakov, O., Barba, F.J., Grimi, N., Marchal, L., Jubeau, S., Lebovka, N., Vorobiev, E., 2015. Pulsed electric field and pH assisted selective extraction of intracellular components from microalgae *Nannochloropsis*. Algal Res. 8, 128–134.

Patil, G., Chethana, S., Madhusudhan, M.C., Raghavarao, K.S.M.S., 2008. Fractionation and purification of the phycobiliproteins from *Spirulina platensis*. Bioresour. Technol. 99, 7393–7396.

Pazour, G.J., Witman, G.B., 2009. The *Chlamydomonas* flagellum as a model for human ciliary disease. In: Witman, G.B. (Ed.), The *Chlamydomonas* Sourcebook (Vol. 3, Cell Motility and Behavior). Elsevier, pp. 445–478.

Petrusevski, B., Bolier, G., Van Breemen, A.N., Alaerts, G.J., 1995. Tangential flow filtration: a method to concentrate freshwater algae. Water Res. 29 (5), 1419–1424.

Pignolet, O., Jubeau, S., Vaca-Garcia, C., Michaud, P., 2013. Highly valuable microalgae: biochemical and topological aspects. J. Ind. Microbiol. Biotechnol. 40, 781–796.

Plaza, B.M., Gómez-Serrano, C., Acién-Fernández, F.G., Jimenez-Becker, S., 2018. Effect of microalgae hydrolysate foliar application (*Arthrospira platensis* and *Scenedesmus* sp.) on Petunia x hybrida growth. J. Appl. Phycol. 30, 2359–2365.

Prabakaran, P., Ravindran, A., 2011. A comparative study on effective cell disruption methods for lipid extraction from microalgae. Lett. Appl. Microbiol. 53, 150–154.

Raja, R., Hemaiswarya, S., Kumar, N.A., Sridhar, S., Rengasamy, R., 2008. A perspective on the biotechnological potential of microalgae. Crit. Rev. Microbiol. 34 (2), 77–88.

Raja, R., Hemaiswarya, S., Ganesan, V., Carvalho, I.S., 2016. Recent developments in therapeutic applications of cyanobacteria. Crit. Rev. Microbiol. 42 (3), 394–405.

Raja, R., Coelho, A., Hemaiswarya, S., Kumar, P., Carvalho, I.S., Alagarsamy, A., 2018. Applications of microalgal paste and powder as food and feed: an update using text mining tool. Beni-Suef Univ. J. Basic Appl. Sci. 7 (4), 740–747.

Rajendran, N., Puppala, S., Sneha Raj, M., Ruth Angeeleena, B., Rajam, C., 2012. Seaweeds can be a new source for bioplastics. J. Pharm. Res. 5, 1476–1479.

Ramaraj, S., Ramalingam, R., Abeer, H., Elsayed, F.A., 2017. Microalgae metabolites: a rich source for food and medicine. Saudi J. Biologic. Sci. 26 (4), 709–722.

Ranga Rao, A., Phang, S.M., Ravi, S., Aswathanarayana, R.G., 2014. Astaxanthin: sources, extraction, stability, biological activities and its commercial applications - a review. Mar. Drugs 12 (1), 128–152.

Regnier, P., Bastias, J., Rodriguez-Ruiz, V., Caballero-Casero, N., Caballo, C., Sicilia, D., Fuentes, A., Maire, M., Crepin, M., Letourneur, D., Gueguen, V., Rubio, S., Pavon-Djavid, G., 2015. Astaxanthin from *Haematococcus pluvialis* prevents oxidative stress on human endothelial cells without toxicity. Mar. Drugs 13, 2857–2874.

Richmond, A., 2004. Handbook of Microalgal Culture: Biotechnology and Applied Phycology. Blackwell Science, Oxford, UK.

Rizwan, M., Mujtaba, G., Memon, S.A., Lee, K., Rashid, N., 2018. Exploring the potential of microalgae for new biotechnology applications and beyond: a review. Renew. Sust. Energ. Rev. 92, 394–404.

Rolland, N., Atteia, A., Decottignies, P., Garin, J., Hippler, M., Kreimer, G., L, S.D., Mittag, M., Wagner, V., 2009. *Chlamydomonas* proteomics. Curr. Opin. Microbiol. 12, 285–291.

Ronga, D., Biazzi, E., Parati, K., Carminati, D., Carminati, E., Tava, A., 2019. Microalgal biostimulants and biofertilisers in crop productions. Agronomy 9 (4), 192.

Safafar, H., van Wagenen, J., Møller, P., Jacobsen, C., 2015. Carotenoids, phenolic compounds and tocopherols contribute to the antioxidative properties of some microalgae species grown on industrial wastewater. Mar. Drugs 13 (12), 7339–7356.

Safi, C., Zebib, B., Merah, O., Pontalier, P.-Y., Vaca-Garcia, C., 2014. Morphology, composition, production, processing and applications of *Chlorella vulgaris*: a review. Renew. Sust. Energ. Rev. 35, 265–278.

Sajilata, M.G., Singhal, R.S., Kamat, M.Y., 2008. The carotenoid pigment zeaxanthin - a review. Comp. Rev. Food Sci. Food Saf. 7 (1), 29–49.

Sakarika, M., Kornaros, M., 2019. *Chlorella vulgaris* as a green biofuel factory: comparison between biodiesel, biogas and combustible biomass production. Bioresour. Technol. 273, 237–243.

Sathasivam, R., Radhakrishnan, R., Hashem, A., Abd Allah, E.F., 2019. Antioxidant potential of microalgae in relation to their phenolic and carotenoid content. Saudi J. Biol. Sci. 26 (4), 709–722.

Shaaban, M.M., 2001. Green microalgae water extract as foliar feeding to wheat plants. Pakistan J. Biol. Sci. 4 (6), 628–632.

Shah, M.R., Lutzu, G.A., Alam, A., Sarker, P., Chowdhury, M.A.K., Parsaeimehr, A., Liang, Y., Daroch, M., 2018. Microalgae in aquafeeds for a sustainable aquaculture industry. J. Appl. Phycol. 30, 197–213.

Shao, Y., Pan, J., Zhang, C., Jiang, L., He, Y., 2015. Detection in situ of carotenoid in microalgae by transmission spectroscopy. Comput. Electron. Agric. 112, 121–127.

Shumbulo, E., Ki, D., 2018. Microalgae to biofuels: promising alternative and renewable energy, review. Renew. Sustain. Energ. Rev. 81, 743–755.

Shuping, Z., Yulong, W., Mingde, Y., Kaleem, I., Chun, L., Tong, J., 2010. Production and characterization of bio-oil from hydrothermal liquefaction of microalgae *Dunaliella tertiolecta* cake. Energy 35, 5406–5411.

Silflow, C.D., Lefebvre, P.A., 2001. Assembly and motility of eukaryotic cilia and flagella. Lessons from *Chlamydomonas reinhardtii*. Plant. Physiol. 127, 1500–1507.

Silva, J., Alves, C., Pinteus, S., Reboleira, J., Pedrosa, R., Bernardino, S., 2019. Chapter 3.10 - *Chlorella*. In: Nonvitamin and Nonmineral Nutritional Supplements, pp. 187–193.

Singh, N.K., Dhar, D.W., 2011. Microalgae as second generation biofuel. A review. Agro. Sust. Dev. 31, 605–629.

Soni, R.A., Sudhakar, K., Rana, R.S., 2017. *Spirulina* – from growth to nutritional product: a review. Trends Food Sci. Technol. 69, 157–171.

Spolaore, P., Joannis-Cassan, C., Duran, E., Isambert, A., 2006. Commercial applications of microalgae. J. Biosci. Bioeng. 101 (2), 87–96.

Stolz, P., Obermayer, B., 2005. Manufacturing microalgae for skin care. Cosmetics Toilet 120, 99–106.

Suparmaniam, U., Lam, M.K., Uemura, Y., Lim, J.W., Lee, K.T., Shuit, S.H., 2019. Insights into the microalgae cultivation technology and harvesting process for biofuels production: a review. Renew. Sust. Energ. Rev. 115, 109361. https://doi.org/10.1016/j.rser.2019.109361.

Sutton, M.A.B.A., Howard, C.M., Bekunda, M.G.B., de Vries, W., van Grinsven, H.J.M., Abrol, Y.P., Adhya, T.K., Billen, G., Davidson, E.A., Datta, A., Diaz, R., Erisman, J.W., Liu, X.J., Oenema, O., Palm, C., Raghuram, N., Reis, S., Scholz, R.W., Sims, T., Westhoek, H., Zhang, F.S., 2013. Our Nutrient World: The Challenge to Produce More Food and Energy With Less Pollution. NERC/Centre for Ecology & Hydrology, Edinburgh, pp. 114.

Tandon, P., Jin, Q., 2017. Microalgae culture enhancement through key microbial approaches. Renew. Sust. Energ. Rev. 80, 1089–1099.

Tang, H., Abunasser, N., Garcia, M.E.D., Chen, M., Simon, K.Y., Salley, S.O., 2011. Potential of microalgae oil from *Dunaliella tertiolecta* as a feedstock for biodiesel. Appl. Energy 88, 3324–3330.

Tasic, M.B., Pinto, L.F.R., Klein, B.C., Veljkovic, V.B., Filho, R.M., 2016. *Botryococcus braunii* for biodiesel production. Renew. Sust. Energ. Rev. 64, 260–270.

Uysal, O., Uysal, F.O., Ekinci, K., 2015. Evaluation of microalgae as microbial fertilizer.

Eur. J. Sustain. Dev. 4 (2), 77–82.
Vandamme, D., Foubert, I., Muylaert, K., 2013. Flocculation as a low-cost method for harvesting microalgae for bulk biomass production. Trends Biotechnol. 31, 233–239.
Varfolomeev, S.D., Wasserman, L.A., 2011. Microalgae as source of biofuel, food, fodder and medicine. Appl. Biochem. Microbiol. 47, 789–807.
Velichkova, K., Sirakov, I., Georgiev, G., 2012. Cultivation of *Botryococcus braunii* strain in relation of its use for biodiesel production. J. BioSci. Biotech. SE/ONLINE 157–162.
Verdugo, M., Lim, L.T., Rubilar, M., 2014. Electrospun protein concentrate fibers from microalgae residual biomass. J. Polym. Environ. 22 (3), 373–383. https://doi.org/10.1007/s10924-014-0678-3.
Vilchez, C., Garbayo, I., Lobato, M.V., Vega, J.M., 1997. Microalgae-mediated chemicals production and wastes removal. Enzym. Microb. Technol. 20, 562–572.
Villar, R., Laguna, M.R., Calleja, J.M., Cadavid, I., 1992. Effects of *Skeletonema costatum* extracts on the central nervous system. Planta Med. 58, 405–409.
Volkman, J.K., 2014. Acyclic isoprenoid biomarkers and evolution of biosynthetic pathways in green microalgae of the genus *Botryococcus*. Org. Geochem. 75, 36–47.
Wake, L.V., Hillen, L.W., 1980. Study of a "bloom" of the oil-rich alga *Botryococcus braunii* in the Darwin river reservoir. Biotechnol. Bioeng. 22, 1637–1656.
Walker, T.L., Purton, S., Becker, D.K., 2005. Microalgae as bioreactors. Plant Cell Rep. 24, 629–641.
Wang, L., Wang, Y., Chen, P., Ruan, R., 2010. Semi-continuous cultivation of *Chlorella vulgaris* for treating undigested and digested dairy manures. Appl. Biochem. Biotechnol. 162, 2324–2332.
Wang, M., Yuan, W., Jiang, X., Jing, Y., Wang, Z., 2014. Disruption of microalgal cells using high-frequency focused ultrasound. Bioresour. Technol. 153, 315–321.
Weiss, T.L., Johnston, J.S., Fujisawa, K., Okada, S., Devarenne, T.P., 2011. Genome size and phylogenetic analysis of the a and L races of *Botryococcus braunii*. J. Appl. Phycol. 23, 833–839.
WEO/IPCS, 1993. Toxicological evaluation of certain food additives and contaminants. In: International Programme on Chemical Safety (IPCS). World Health Organization (WEO). https://apps.who.int/iris/handle/10665/41742.
Wolf, F.R., Nonomura, A.M., Bassham, J.A., 1985. Growth and branched hydrocarbon production in a strain of *Botryococcus braunii* (Chlorophyta). J. Phycol. 21, 388–396.
Yaakob, Z., Ali, E., Zainal, A., Mohamad, M., Takriff, M.S., 2014. An overview: biomolecules from microalgae for animal feed and aquaculture. J. Biol. Res. Thessalon. 21 (1), 6.
Yamaguchi, K., 1997. Recent advances in microalgal bioscience in Japan, with special reference to utilization of biomass and metabolites: a review. J. Appl. Phycol. 8, 487–502.
Yamamoto, M., Fujishita, M., Hirata, A., Kawano, S., 2004. Regeneration and maturation of daughter cell walls in the autospore-forming green alga *Chlorella vulgaris* (Chlorophyta, Trebouxiophyceae). J. Plant Res. 117, 257–264.
Yates, M.R., Barlow, C.Y., 2013. Life cycle assessments of biodegradable, commercial biopolymers: a critical review. Resour. Conserv. Recy. 78, 54–66. https://doi.org/10.1016/j.resconrec.2013.06.010.
Ye, Z.-W., Jiang, J.-G., Wu, G.-H., 2008. Biosynthesis and regulation of carotenoids in *Dunaliella*: progresses and prospects. Biotechnol. Adv. 26, 352–360.
Yoo, G., Park, W.-K., Kim, C.W., Choi, Y.-E., Yang, J.-W., 2012. Direct lipid extraction from wet *Chlamydomonas reinhardtii* biomass using osmotic shock. Bioresour. Technol. 123, 717–722.
Zeller, M.A., Hunt, R., Jones, A., Sharma, S., 2013. Bioplastics and their thermoplastic blends from *Spirulina* and *Chlorella* microalgae. J. Appl. Polym. Sci. 130, 3263–3275.
Zenouzi, A., Ghobadian, B., Hejazi, M.A., Rahnemoon, P., 2013. Harvesting of microalgae *Dunaliella salina* using electroflocculation. Cold Reg. Sci. Technol. 15 (5), 879–887.
Zhang, C., Show, P.L., Ho, S.H., 2019. Progress and perspective on algal plastics – a critical review. Bioresour. Technol. 289, 121700. https://doi.org/10.1016/j.biortech.2019.121700.
Zhang, C., Wang, C., Cao, G., Wang, D., Ho, S.H., 2020. A sustainable solution to plastics pollution: An eco-friendly bioplastics film production from high-salt contained *Spirulina* sp. residues. J. Hazard. Mater. 388, 121773. https://doi.org/10.1016/j.jhazmat.2019.121773.
Zhu, L., Li, Z., Hiltunen, E., 2018. Microalgae *Chlorella vulgaris* biomass harvesting by natural flocculant: effects on biomass sedimentation, spent medium recycling and lipid extraction. Biotechnol. Biofuels 11. https://doi.org/10.1186/s13068-018-1183-z.
Zullaikah, S., Jessinia, M.C.P., Rinaldi, Yasmin, Rachimoellah, M., Wu, D.W., 2019. Lipids extraction from wet and unbroken microalgae *Chlorella vulgaris* using subcritical water. Mat. Sci. Forum. 964, 103–108.