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VALORIZATION OF MICROALGAL BIOMASS

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Abstract

Microalgae can be considered as significant sources of sustainable and beneficial biocompounds such as carotenoids, lipids, proteins, polysaccharides, pigments, vitamins etc., with many uses in energy production (biofuels), pharmaceutical, cosmetic and food industries. This class of microorganisms are considered as sustainable feedstock due to some major advantages related to metabolism modulation, faster growth rate compared to plants, no use of terrestrial land. This review will present the most important bioactive compounds founded in microalgae, with high potential impact in industry and the technologies used for cultivation and downstream processes.

*Key words:*biomass, bioproducts, microalgae, valorisation

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1. Introduction

Microalgae are a large and very diverse group of photosynthetic microorganisms, characterised by some features, that make them extremely interesting for food, cosmetic, pharmaceutical industries and agriculture, because they have high growth rates, do not require soil or fertilizer and pesticides, they can be grown all the year depending on the climate, have the ability to fix CO_2 with higher rate than the terrestrial plants, and are highly biodegradable (Jankowska et al., 2017; Luangpipat and Chisti, 2017).

Algae are an important resource for numerous natural products used in different industries (Table 1), some with exceptionally high market values: algal biomass of *Chlorella* and *Spirulina* costs about 40 to 50 US-$/kg for human nutrition or 10 US-$/kg, for animal feeding and aquaculture; the price of microalgal ß-carotene is estimated at 300 to 3000 US-$/kg and astaxanthin about 2500 to 7000 US-$/kg determined by the purity (Koller et al., 2014).

1.1. Growth systems

The chemical composition of algae is very different in its main components (proteins, carbohydrates and lipids), depending on the species (Table 2), availability of nutrients, and environmental factors.

The selection of an appropriate strain of microalgae, which can be used for food, feed, or biofuel production, must take into account several characteristics (Rajauria et al., 2015):

- can be cultivated constantly and continuously either in an open pond or a closed photobioreactor (PBR);
- produces high and constant quantities of desirable bioactive compounds;

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- survives and grows in spite of seasonal differences and daily climate changes;
- produces biomass at a large scale;
- provides high photosynthetic efficiency and energy conversion rate;
- generates minimal fouling from attachment to the sides or bottom of the production vessel;
- it can be easy harvested and submitted to different methods of extraction (soft or flexible cell walls).

Table 1. Bioactive compounds separated from microalgae (Priyadarshani and Rath, 2012; Raposo et al., 2013)

Product group	***Application***	***Product***	***Algae***
Pigments/ carotenoids	Cosmetics, Food industry, Pigmentation	β-carotene	*Dunaliella salina, Nannochloropsis gaditana*
		Astaxanthin, Lutein Canthaxanthin	*Haematococcus pluvialis, Chlorella vulgaris*
Polyunsaturated fatty acids	Food additives	Eicosapentaenoic acid, omega-3, docosahexaenoic acid	*Chlorella minutissima, Phaeodactylum tricornutum, Porphyridium cruentum*
		Arachidonic acid	*Parietochlorisincise, Porphyridium cruentum*
		Docosahexanoic acid	*Schizochytrium sp.*
		γ-Linoleic acid	*Arthrospira, Porphyridium*
Vitamins	Nutrition	Biotin	*Euglena gracilisa*
		Vitamin C	*Prototheca moriformis, Chlorella vulgaris*
		Vitamin B_{12}	*Cylindrospermum sp.,Tolypothrix tenuis, Nostoc muscorum, Spirulina*
		Vitamin D_2	*Chlorella sp., Spirulina*
Sterols	Nutrition	Brassicasterol, stigmasterol	*I. galbana, Chaetoceros, Skeletonema, P. lutheri*
Polysaccharides	Pharmaceuticals	β-glucan, spirulan, homogalactan, (1,4)-D-glucan	*Chlorella vulgaris, Spirulina platensis, Gyrodinium impudicum, Porphyridium cruentum, Dunaliella tertiolecta*
Enzymes	Nutrition, Pharmaceuticals	Carbonic anhydrase	*I. galbana, Amphidinium carterae, Prorocentrum minimum*
		Superoxide dismutase (SOD)	*P. tricornutum, Porphyridium, Anabaena, Synechococcus*

Table 2. Microalgae composition (Priyadarshani et al., 2012; Rajauria et al., 2015)

Microalgae strain	***Proteins, % d.w.***	***Carbohydrates, % d.w.***	***Lipids, % d.w.***
High amounts of proteins			
Synechococcus sp.	**73**	15	11
Arthrospira maxima	**61 – 71**	13 – 14	6 – 7
Spirulina platensis	**61 – 64**	15 – 16	7 – 8
Spirulina maxima	**60–71**	13–16	6–7
Aphanizomenon flos-aquae	**62**	23	3
Dunaliella salina	**57**	32	6
Scenedesmus obliquus	**50 – 56**	10 – 17	12 – 14
Chlamydomonas rheinhardei	**48**	17	21
Anabaena cylindrica	**43–56**	25–30	4–7
Botryococcus braunii	**40**	2	33
Chlorella pyrenoidosa	**57**	26	2
Chlorella vulgaris	**41–58**	12–17	10–22
High amounts of carbohydrates			
Spirogira sp.	6 – 20	**33 – 64**	11 – 21
Nostoc commune	20.9	**55.7**	1.2
Porphyridium cruentum	28 – 39	**40 – 57**	9 – 14
Scenedesmus dimorphus	8 – 18	**21 – 52**	16 – 40
Navicula sp.	12.7	**46.9**	31.7
Nitzschia sp.	31.4	**36.4**	28.5
High amounts of lipids			
Schizochytrium sp.	13.2	19.4	**50 – 77**
Nannocholoropsis sp.	18 – 46	3	**31 – 68**
Botrycoccus braunii	5 – 45	15 – 20	**35 – 75**
Neochloris oleoabundans	26 – 42	4 – 39	**35 – 54**

The main nutritional factors that influence the microalgae growth are the C-source, the N-source, the minerals, trace-elements (iron, manganese, molybdenum, nickel) and vitamins, and the manipulation of these factors can increase biomass productivity. In many strains the nitrogen: phosphorus ratio plays a significant role in both biomass and hydrocarbon production, while the trace elements play critical roles in the metabolic pathways involving the utilization of light, nitrogen, phosphorus, and CO_2. Different nutrient concentration (e.g., nitrogen, phosphorus, sulphate, calcium), high salinity, high temperature, light intensity, and alternative sources of organic carbon can induce stress in microalgae cultivation that will increase the accumulation of some compounds but reducing the growth rate simultaneously.

For the production of the bioactive compounds it is important to consider not only microalgae cultivation but also the processes used for separation and purification, both being highly expensive and requiring intensive energy consumption. The microalgae's bioactive compound composition is strongly influenced by species, location, growing conditions but also by the composition of the culture medium and the conditions used for growth (nutritional stress: reduction of nitrogen or phosphate content in the culture media, the presence of salts, strong light conditions) (Fig. 1) (Raposo et al., 2013; Silva Vaz et al., 2016).

Considering the cultivation systems (Fig. 2), microalgae can be grown in open ponds or open basins (Cheah et al., 2016) and closed photobioreactors with different configurations (Fig. 3), but also in hybrid systems (two types of reactors are used for two different growth stages) currently used in research (pilot and laboratory).

Algae culturing in one of these systems requires analysis of both configurations (Table 3) and selection of the most appropriate one in relation to their properties. Microalgae are able to grow and develop in very different conditions, but in order to obtain high productivity and the consistency of the structure and composition of the bioactive compounds, it is important to have a very good control of the process.

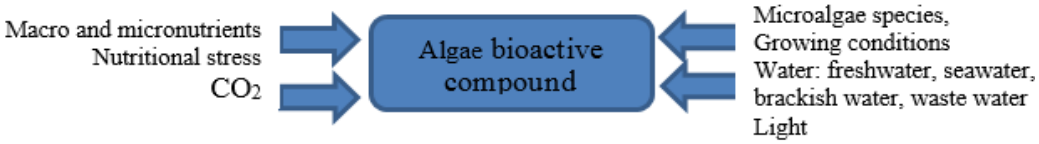


Fig. 1. Factors that influence microalgae growth and composition

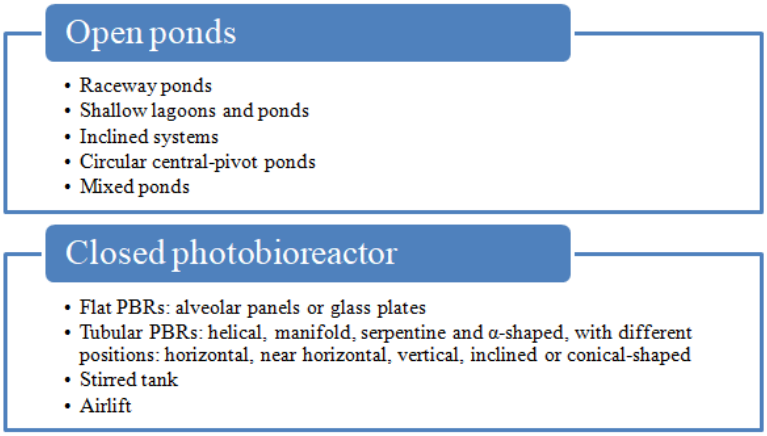
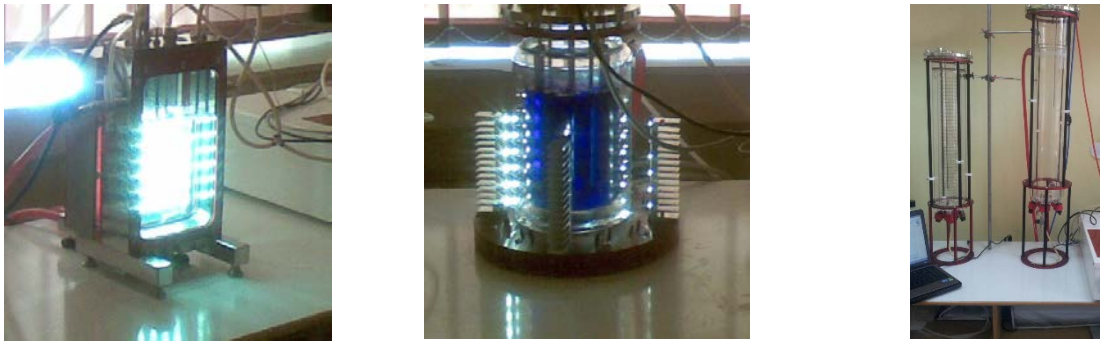


Fig. 2. Main systems for cultivation of microalgae



A B C

Fig. 3. Closed photobioreactors: A - Flat-vessel; B – Stirred tank; C – Airlift column

Table 3. Comparison between open ponds and closed photobioreactors (Blaga et al., 2016; Cascaval et al., 2007)

Open ponds		***Closed photobioreactors (PBRs)***	
Advantages	***Disadvantages***	***Advantages***	***Disadvantages***
- lower cost compared to PBRs; - easier to build and to operate than PBRs.	- low productivity; - high contamination risk; - low gas-liquid masstransfer rate; - huge water evaporation; - poor mixing, controland light penetration; - require large area ofland and high harvesting cost.	- low contamination risk; - control and enhancement of gas-liquid mass transfer rate; - reduction of water loss; - high biomass density - high volumetric and areal productivity; - low harvesting costs.	- low light penetration; - oxygen inhibition due to its accumulation in the tubes; - difficult to scale up; - sophisticatedconstruction.

Table 4. Content of bioactive compounds in microalgae (Ahmed et al., 2014; Foo et al., 2017; Goiris et al., 2012; Koller et al., 2014; Kent et al., 2015; Maadane et al., 2015; Morais et al., 2015; Rodrigues et al., 2015; Sloth et al., 2006)

Bioactive compound	***Concentration***	***Algae strain***
C-phycocyanin	46.0 % w/w	*Spirulina fusiformis*
	15–28 mg/g d.w.	*Galdieria sulphuraria 074G*
Alkaloids	3.02 % w/w	*Spirulina platensis*
Terpenoids	0.14 % w/w	*Spirulina platensis*
Lutein	6.55 ± 0.92 mg/g	*Dunaliella salina*
	128.56 ± 0.5 µg/g d.w.	*Phormidium autumnale*
Zeaxanthin	11.27 ± 1.58 mg/g	*Dunaliella salina*
	88.46 µg/g	*Phormidium autumnale*
β-carotene	138.25 ± 10.03 mg/g	*Dunaliella salina*
	225.44 µg/g	*Phormidium autumnale*
Astaxanthin	6.4 ± 1.2 mg/g	*Nannocholopsis sp.*
Linoleic acid	8.43 ± 3.72 mg/g	*Dunaliella sp.*
Arachidonic acid	7.45 ± 1.79 mg/g	*Nannocholopsis sp.*
Eicosapentaenoic acid	36.76 ± 2.93 mg/g	*Nannocholopsis sp.*
Carotenoids	5.8 mg/g d.w.	*Tetraselmis sp.*
	1.1 mg/g d.w.	*Dunaliella tertiolecta*
	5.0 mg/g d.w.	*Isochrysis sp.*
	6.1 mg/g d.w.	*Phaeodactylum tricornutum*
	2.2 mg/g d.w.	*Nannochloropsis sp.*
Fucoxanthin	2.33 ± 0.44 mg/g d.w.	*Chaetoceros calcitrans:*
	2.19 ± 0.02 mg/g d.w.	*Isochrysis galbana*
	1.18 ± 0.00 mg/g d.w.	*Odontellasinensis*
Poly(hydroxyalkanoates) (PHA)	77 % d.w	*Aulosira fertilissima*
	55 % d.w	*Synechococcus sp. MA19*

Recently, researchers worldwide have been investigating cultivation systems based on the use of wastewater, trying to achieve waste reduction and renewable energy production in one-step. Such studies used species like: *Chlorella* and *Scenedesmus*, for biofuel production (Cheah et al., 2016). Effective bioreactors design needs to be focused on minimizing energy consumption, increasing productivity, and reducing total cost of the production process (Manirafasha et al., 2016).

Over the last years, photobioreactor design has evolved especially for improved control and for long-term stability and reliability of operations. In order to choose the most appropriate bioreactor it is necessary to consider not only the yield, but also the increase in photosynthetic efficiency and enhancement of gas exchange rate, as well as the capital investment and operating costs, for a commercially feasible choice. Many challenges are still to be overcome in investigating models for radiative transfer mechanism, hydrodynamics, but also for photosynthetic and growth kinetics (Hallenbeck et al., 2016; Lamand Lee, 2012; Olivieri et al., 2014). Efficient large scale cultivation, but also appropriate techniques for harvesting and post-processing are necessary for providing a feasible commercial manufacture process.

2. Bioactive compounds

Microalgae are highly efficient photosynthetic species fast-growing, very rich in bioactive compounds (Table 4) that can be used in several applications related with health benefits.

The following microalgae: *Spirulina*, *Chlorella*, *Dunaliella*, *Nostoc*, *Botryococcus*, *Haematococcu*s (Koller et al., 2014; Silva Vaz et al., 2016), are grown at commercially scale for food supplements.

2.1. Carotenoids

There is an increasing interest in finding natural, safe and powerful antioxidants in order to minimize oxidative damage to living cells and prevent

oxidative deterioration in commercialized products such as food, pharmaceuticals or cosmetics. The major carotenoids that were obtained and tested for human use are: β-carotene, astaxanthin, lutein, lycopene, fucoxanthin and canthaxanthin (Vigani et al., 2015).

Microalgae have a higher specific carotenoid content (compared to terrestrial plants). Species like: *Dunaliella salina*, *Haematococcus pluvialis*, *Porphyridium cruentum*, *Chlorella vulgaris*, *Chlorella zofingiensis* and *Chlorella pyrenoidosa* have been successfully used for the mass production of β-carotene, astaxanthin, canthaxanthin, lutein and other carotenoids (Gong and Bassi, 2016). Even if the use of microalgae as a source of carotenoids (given their antioxidant activity, nutritional value and as a food colorant) is justified by many advantages of this process: no requirement for storage or potential degradation of carotenoids in time, due to the possibility to grow algae during all year and the fact that synthetic carotenoids (cheaper) were incriminated for several undefined diseases: β-carotene and lung cancer (Goralczyk, 2009; Omenn, 1996), the cost of the process, mainly related to the harvesting and post-processing, is still very high (Foo et al., 2017). The diagram of the process is presented in Fig. 4.

Carotenoids can be divided into primary carotenoids (growth-coupled metabolites): lutein, β-carotene (but under stress conditions act as secondary metabolite) and secondary carotenoids: astaxanthin. For the primary carotenoids a cultivation process in one step is recommended, due to the degradation of carotenoids under stress (lutein, for example), while for the secondary carotenoids, like astaxanthin or β-caroten, the recommended process implies two steps: in the first one the optimum conditions for growth and multiplication are provided, while the second step will be realised under extreme stress conditions (reduction of nitrogen, phosphate, or the presence of salts: sodium chloride or ferrous salts, strong light conditions).

The downstream part includes harvesting the cells by different physical and chemical methods (Fig. 4), with a cost contribution in the overall process of 20-30%. Flocculation has attracted much interest recently due to some major advantages: the ability to treat large scale suspensions with low cost and less energy consumption (Gong and Bassi, 2016). Cell disruption, due to the presence of a thick rigid cell wall, is often suggested as a necessary step to increase the carotenoids recovery yield by several times. Even if it introduces additional processing cost, the pre-treatment step is still viewed as mandatory. The methods used for this step could be of mechanical and non-mechanical type.

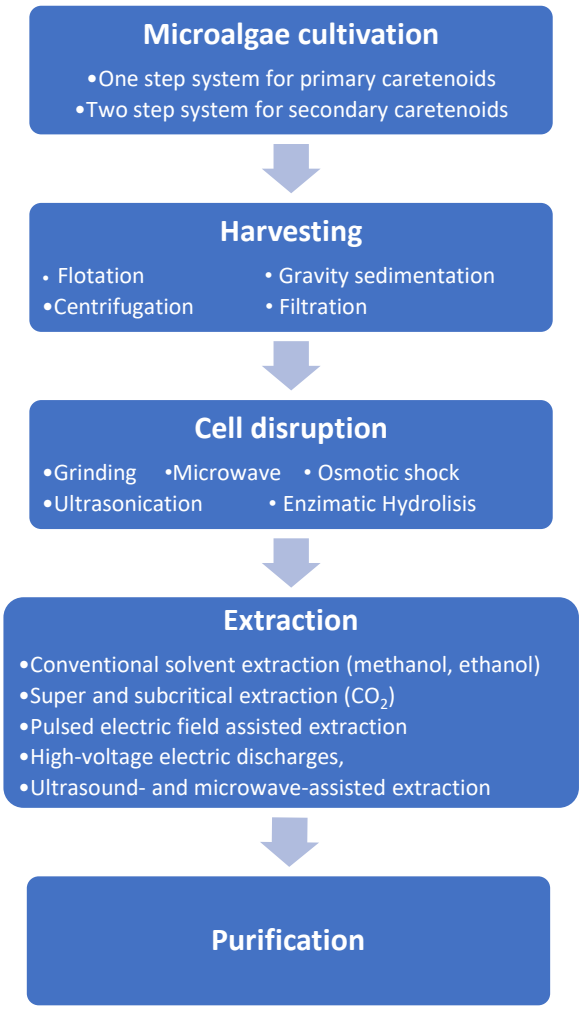


Fig. 4. Process diagram of carotenoids production

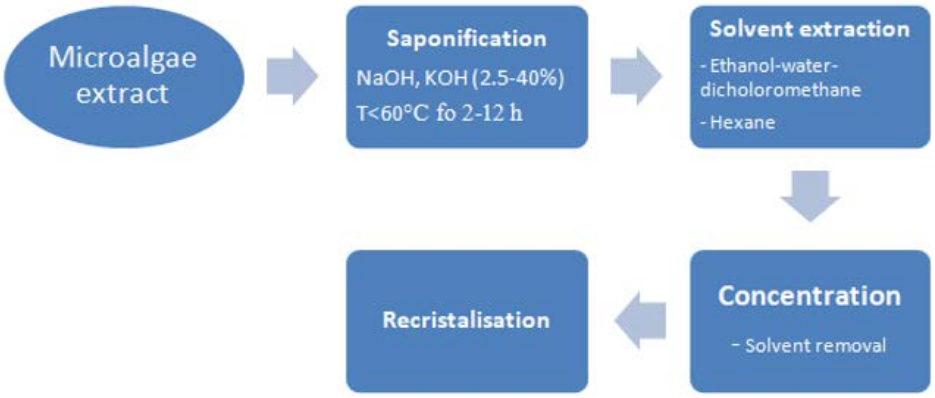


Fig. 5. Process diagram of carotenoids purification

Mechanical methods including high pressure homogenisers, grinding and bead milling are easy to scale up, but have high energy requirements (Taucher et al., 2016), while the non-mechanical one, like microwave, ultrasonication or enzymatic hydrolysis have great potential requiring less energy. However, the non-mechanical methods present some drawbacks: the first two needs a very efficient temperature control as the carotenoids start to degrade at 60°C and the last one is quite expensive due to enzyme price (Kim et al., 2015). Due to the lack of specificity of these cell disruption methods that causes the release of cell debris or other impurities, a pulsed electric fields (PEF) assisted extraction has been investigated for the extraction of nutritionally valuable compounds (total chlorophylls, carotenoids, proteins, phenolic compounds) from microalgae *Nannochloropsis sp.* using the binary mixture of organic solvents (dimethyl sulfoxide, DMSO and ethanol, EtOH) and water (Parniakov et al., 2015a). Pulsed electric fields (PEF) can increase the cell membrane permeability (electroporation), by high-intensity electric field pulses of short duration (microseconds to milliseconds) and can facilitate the purification steps, and also minimizing the energetic costs and losses due to the drying of the biomass (Barba et al., 2015; Parniakov et al., 2015b).

Carotenoids extraction can be achieved by conventional solvent extraction, but recently supercritical solvents (especially CO_2) are investigated, due to the possibility to conduct a process that avoids heat-degradation of active compounds, and offers higher selectivity, shorter extraction times and does not use toxic organic solvents (Millao and Uquiche, 2016a). Trying to overcome the limitations of batch operation related to high-pressure conditions, ways to increase the load of extraction equipment were investigated, namely: pelletization of the microalgae, method that also prevents filter clogging (Millao and Uquiche, 2016a).

Carotenoids purification process is based on a multi-step Willstatter method (Fig. 5), but recently other techniques were investigated: selective absorption, supercritical anti-solvent precipitation, expanded bed coupled column chromatography or reversed phase HPLC (Gong and Bassi, 2016).

Maadane et al. (2015) performed the extraction of bioactive compounds from nine marine microalgae: *Nannochlorpsis gaditana, Dunaliella sp., Dunaliella salina, Phaedactylium tricornutum, Isochrysis sp., Navicula sp., Chaeotoceros sp., Chlorella sp.* and *Tetraselmis sp.*, grown in sterile natural seawater using conventional solvent extraction with ethanol, water and a mixture of ethanol and water, for different polarities. The authors obtained higher antioxidant activities for the ethanolic extracts of all tested microalgae: *Dunalliela sp.* - 10.8 mg/g carotenoids and 14.5 mg/g phenolic content, *Tetraselmis sp.* – 4.6 mg/g carotenoids and 25.5 mg/g phenolic content and *Nannochloropsis gaditana* – 3.02 mg/g carotenoids and 32.0 mg/g phenolic content (Maadane et al., 2015).

Foo et al. (2017) have investigated the carotenoids extraction in several tropical microalgae species: *C. calcitrans, Isochrysis galbana, Saccharina japonica, Skeletonema costatum, Odontella sinensis, Phaeodactylum tricornutum* using methanol as a solvent. Methanol has the most suitable polarity in extracting antioxidants especially from brown microalgae (Foo et al., 2015). Thus, they obtained the highest amount of carotenoids (6.13 ± 0.25 mg/g d.w.) and fucoxanthin (5.13 ± 0.19 mg/g d.w.) in the extract from *C. calcitrans*, followed by 4.33 ± 0.04 mg/g d.w. carotenoids and 2.19 ± 0.02 mg/g d.w. fucoxanthin in *I. galbana*, and 2.66 ± 0.10 mg/g d.w. carotenoids for *O. sinensis*.

Millao and Uquiche (2016) studied the effects of temperature (36 – 64°C) and CO_2 density (914 – 956 kg/m^3) on the content of carotenoids from *N. gaditana* with a moisture content of 30%. The performance of the process increased with CO_2 density and temperature, the highest values for antioxidant activities being obtained at 64°C and CO_2 density of 956 kg/m^3 (Millao and Uquiche, 2016b).

Rodrigues et al. (2015) obtained twenty-four carotenoids, being the most important: all-trans-β-carotene (225.44 μg/g), all-trans-lutein (117.56 μg/g) and all-trans-zeaxanthin (88.46 μg/g) from microalgae *Phormidium autumnale* grown in a bubble column photobioreactor, separated by centrifugation and using ethyl acetate and methanol as solvents for carotenoids extraction.

2.2. Phycobiliproteins - Phycocyanin

Phycocyanin is a photosynthetic blue pigment, water soluble, highly fluorescent and a major antioxidative protein from the phycobiliprotein family, composed of an α- and a β-subunit with 1 (α) and 2 (β) phycocyanobilin groups attached covalently. Commercially, phycocyanin is produced using the cyanobacterium *Spirulina platensis* (blue green algae with a long, thin, monocellular structure) and sunlight as energy source. Beside these microalgae, species of *Galdieria sulphuraria* (phycocyanin shows similar properties to cyanobacterial one and can be purified to the same standards), *Aphanizomenon flosaquae*, *Pyrophyridium sp.* (maximum concentration obtained was 10.2 % d.w. using a two variables experimental design: light and $NaHCO_3$ feeding and proving that irradiance is a determining factor in the high phycobiliprotein accumulation), *Synechocystis sp.* (a maximum yield of 12% d.w. was obtained in BG-11 medium at optimized conditions of pH 10 and 16 h light), *Phormidium ceylanicum* (purity 4.58 obtained by freezing and thawing method), *Limnothrix sp.*, *S. lividus*, *Nostoc sp.*(maximum yield 0.13 g/g d.w. was obtained for the folowing optimum conditions: initial pH 8, light intensity: 40 µmol/m^2 s, temperature 35°C, diurnal cycle of 16:8 h (light:dark regime), 75.48 µM Na_2CO_3 and 17.65 mM $NaNO_3$)and *Arthronema africanum* were also studied (Johnson et al., 2014; Manirafasha et al., 2016; Martínez et al., 2017; Nakagawa et al., 2016).

Phycocyanin properties proved important activities in medicine and pharmaceuticals: antioxidant, anti-inflammatory, anti-tumor, immunomodulating, atheroprotective, hepatoprotective, neuroprotective, antiviral and antifungal (Manirafasha et al., 2016). The cultivation of a microalgae strain that has the potential to offer high phycocyanin content requires choosing an appropriate system: open ponds or closed photobioreactor (tubular and flat panel configuration seem to be more effective due to better biomass quality) (Carvalho et al., 2014), and heterotrophic, photoautotrophic and mixotrophic (facilitates faster growth with increased phycobiliproteins accumulation, but is not applicable to all microalgae) conditions. It is also important to control other parameters such as: medium composition (e.g. modified Zarrouks medium, artificial sea water, BG 11 medium, SOT medium), temperature, intensity and quality of light, pH, CO_2 availability, and residence time (Manirafasha et al., 2016). The production of phycocyanin requires two main consecutive steps (Fig. 6): upstream process including microalgae biomass production and phycobiliprotein accumulation and downstream processing including harvesting, separation and purification methods.

The upstream process implies the growth of cells in optimum conditions and the accumulation of high content of phycobiliprotein under stress determined by lower nitrate concentration or moderate light intensity (Rio-Chanona et al., 2015). After the biomass production a step of harvesting/dewatering is required, that can be realized by different methods chosen in relation with the microalgae properties, phycocyanin concentration but also taking into account the cost benefits and environmental impact (flocculation is preferred due to less energy demands and scale up possibility).

Phycocyanin can be separated from dry or wet microalgae, the last being economic (reduces the cost associated with drying) but also productive (dry biomass leads to 50 % lost in phycocyanin). After cell-disruption (the freezing and thawing method was considered a good option, robust, simple, fast and reproducible) phycocyanin is extracted using diluted sodium or potassium phosphate buffer (5 – 50 mM, pH 6.0 – 7.5), followed by precipitation by salting out with ammonium sulphate (Sørensen et al., 2013), and dialysis or gel filtration (for desalting).

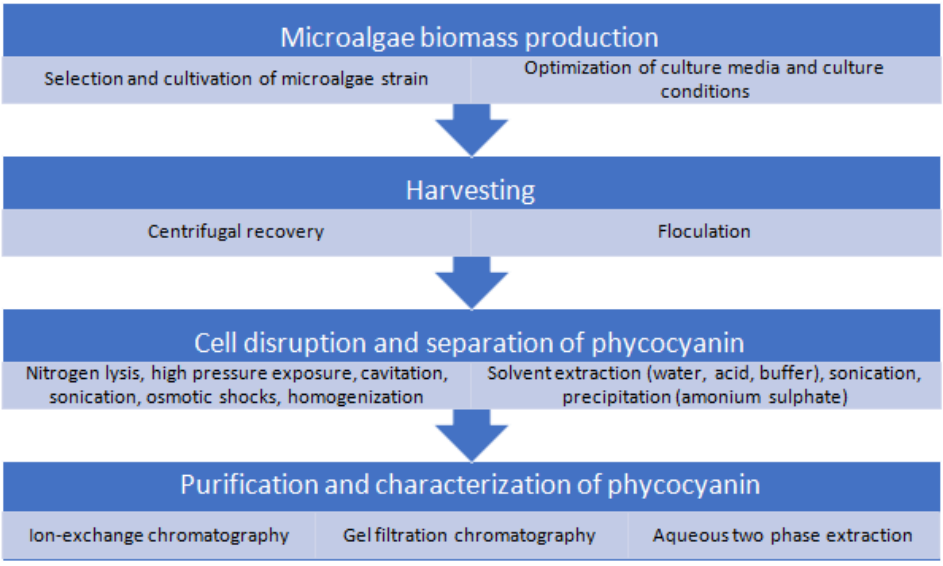


Fig. 6. Process diagram of phycocyanin production

The final step is purification by column chromatography (ion exchange chromatography using DEAE-cellulose DE-52 and hydroxyapatite) from desalted extract. Recently, other methods for separation have been investigated including ultrafiltration, aqueous two phase extraction, ultrasonication, homogenization and different types of chromatography (Chen et al., 2016; Manirafasha et al., 2016), the first offers the advantages of being applicable at large scale and it does not require preliminary stages (precipitation, centrifugation and dialysis).

Chen et al. (2016) used CO_2 feeding, instead of acid/alkaline titration, to control the pH of the culture (pH controlled at 9.5 for *Spirulina platensis* grown in a 1L flat photobioreactor), and a two-stage purification process: fractional precipitation (with $(NH_4)_2SO_4$ 40 %) combined with ion exchange chromatography and obtained a phycocyanin content and productivity enhanced to 16.8 % and 0.17 g/L/d, respectively.

Chaiklahan et al. (2011) investigated a membrane process using microfiltration and ultrafiltration for the purification of phycocyanin from *Spirulina sp*. cultured in Zarrouk's medium in 100 L open raceway ponds (culture depth of 15 cm, outdoor ambient temperatures, mixed with paddle wheels at 12 rpm). After extraction with phosphate buffer and centrifugation they obtained 82 % recovery, 6.17 mg/mL phycocyanin concentration, and 1.07 of the purity ratio. The authors observed a 5 % loss of phycocyanin in sun dried biomass compared to around 80 % loss in oven dried biomass.

Xie et al. (2015) analysed the influence of light intensity and initial biomass concentration on the cultivation of microalgal strain *Spirulina platensis* WH879 in a 1-L photobioreactor (15.5 cm height and 9.5 cm diameter) equipped with an external light source, operated at 28°C, pH 9.0, agitation rate of 400 rpm and 75 – 450 μmol/m^2/s light intensity. The best results were obtained for 300 μmol/m^2/s light intensity and an initial biomass concentration of 0.24 g/L in a fed-batch system: phycocyanin content - 16.1 %, and productivity - 94.8 mg/L/d.

Martinez et al. (2017) studied the application of pulsed electric fields on the extraction of phycocyanin into aqueous media from fresh biomass of *Artrhospira platensis* grown photoautrophically in 2-L bubble column photobioreactor (8 cm diameter and 53 cm height), bubbled with air at 6 mL/s, working at 30°C, in light:dark cycles of 12:12 h with a light intensity of 15 mmol/m^2 s. The maximum amount of extracted phycocyanin (70 % of the total content with a purity of 0.46 ±0.019) was obtained for microalgae under most intensive treatment: 25 kV/cm, 150 μs at 40°C.

2.3. Polysaccharides

Microalgae polysaccharides are renewable materials, biodegradable and generally non-toxic. Due to their properties like water retention capacity, film-forming ability, and rheology they are used as emulsifiers, stabilizers, plant bio-stimulants or thickening agents in a wide variety of industrial applications: food, cosmetics, agriculture, textiles, painting, paper, and pharmaceuticals. Polysaccharides isolated from *Chlorella sp*. have been shown to exhibit strong anti-inflammatory and immunomodulatory properties with important applications in medicine (Chakraborty et al., 2012; Elarroussi et al., 2016). Microalgae can produce two main types of exopolysaccharides: totally released into the environment (that offers an advantage in separation) and associated with the cell surface. The exopolysaccharides are predominantly heteropolysaccharides that include in their structure: sulphates, proteins, pyruvate, methylated sugars and uronic acids.

The main algae strains that can be used for the production of these complex and heterogeneous macromolecules are: *Chlorella vulgaris* – β-glucan, *Spirulina platensis* – spirulan, *Gyrodiniumim pudicum* – homogalactan, *Dunaliella tertiolecta* - (1,4)-D-glucan, but for the majority of microalgae the polysaccharides are divided in sulphated and extracellular. For the cell bound polysaccharides the main strains of microalgae producers are: *Cylindrotheca closterium, Navicula salinarum, Chlorella stigmatophora, Isochrysis sp., Porphyridium sp., Rhodella reticulate, Cochlodinium polykrikoides* (Raposo et al., 2015). For the extracellular polysaccharides, produced in a range from about 0.5 g/L up to 20 g/L, the following microalgae can be used: *Hasleaostrearia, Nitzschia closterium, Skeletonema costatum, Chaetoceros spp., Amphora sp., Dunaliella salina, Ankistrodesmus angustus, Botryococcus braunii, Nostoc sp., Cosmarium sp. etc.* (Delattre et al., 2016).

The biological and pharmacological activities of polysaccharides depend strongly on types of glycosidic linkages and the contents and positions of the sulphate groups which depend on species, but also on growing conditions and extraction procedures (Qi and Kim, 2017). The process for obtaining algal polysaccharides without degradation implies microalgae cultivation, extraction and purification (Fig. 7).

Microalgae cultivation is extremely important for the production of polysaccharides, due to an extremely important effect of the environmental conditions on productivity. Nutrient limitation (nitrogen, sulphate, calcium or phosphorous starvation) is usually used to accumulate large amounts of exopolysaccharides, but this determine a negative effect on cell growth. Since for most exopolysacharides, synthesis occurs along with growth phase (during all growth phases, or just exponential or stationary), research has focused on maximizing different conditions of the culture medium such as: irradiance, carbon dioxide supply, pH and temperature to increase productivity (Delattre et al., 2016).

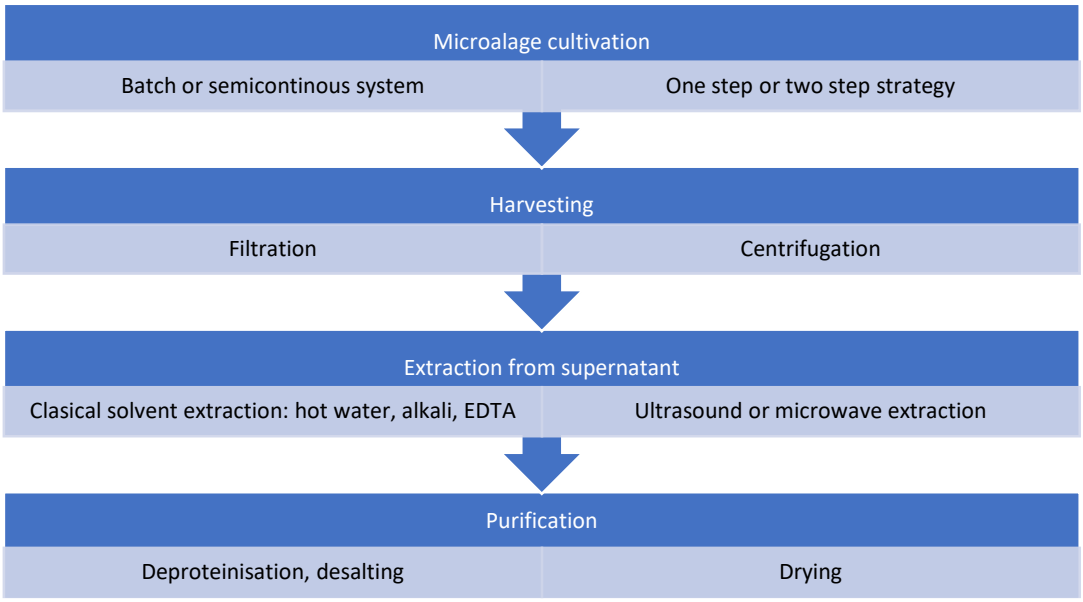


Fig. 7. Process diagram of polysaccharides production

The culture media optimized to increase the polysaccharides production that is usually used for microalgae growth are: BG11, Hemerick, F/2, Provasoli, ASW etc. (Gonzalez-Fernandez and Ballestros, 2013). The downstream part of the process implies the polysaccharides separation using centrifugation or microfiltration to remove microalgae (Li et al., 2011). The extraction of polysaccharides can be made using hot water or alkali solution (the conventional extraction method but with low extraction efficiency), or by microwave or ultrasonic assisted techniques (Patil et al., 2011; Yongjiang et al., 2009) with better performances, shorter time and increased selectively. Next, a precipitation step of the soluble fraction (supernatant or filtrate) using absolute alcohols (methanol, ethanol or isopropanol) at a temperature between –20°C and 20°C is required and followed by desalting by dialysis and membrane techniques.

Qi and Kim (2017) investigated polysaccharides extraction from green alga *Chlorella ellipsoidea*, grown on BG-11 medium at 25°C and 100 µE/m^{2}s illumination with a light/dark cycle of 12:12 h, using hot water and fractionated with anion-exchange chromatography. They analyzed the molecular characteristics of exopolysaccharides that contained 68.1 – 89.7 % carbohydrate, 2.0 – 11.8 % protein, 1.9 – 6.1 % sulphate and 0.5 – 6.1 % uronic acid, with strong immunomodulatory activity.

Chakraborty et al. (2012) analyzed a unique two-step sequential hydrothermal liquefaction process for the simultaneous production of polysaccharides and bio-oil from green alga *Chlorella sorokiniana* (*UTEX 1602*) cultivated in a 5L fermenter. The polysaccharide rich extract (26 % polysaccharides) obtained by a subcritical water extraction at 160°C, was precipitated with ethanol thus proving that concomitant production of value co-products is feasible.

Balavigneswaran et al. (2013) optimized the polysaccharide extraction conditions from microalgae *Isocrysis galbana*, grown using Walne's medium enriched with vitamins: extraction time 3.6 h, temperature 67.02°C, and ratio between water and raw material 10 : 43.

2.4. Peptides

Microalgae biomass contains important quantities of proteins, and due to the fact, that is capable to synthesize all 20 essential amino acids it can become an unconventional source for human nutrition, but also for medicine due to their antihypertensive, antitumoral, antioxidative, and antimicrobial effect. Bioactive peptides (used as growth factors, hormones and immunomodulators) derived from microalgae proteins have natural bioactivities with beneficial health effects on hypertension and oxidative stress, besides the nutritional benefits. These peptides remain inactive until an enzymatic hydrolysis process followed by the isolation of peptides from the enzymatic extracts (process that provides higher yield and purity compared to the organic solvent extractions). The main microalgae strains that could be used for peptides extraction are: *Anabaena cylindrica, Aphanizomenonflos aquae, Arthospira maxima, Chlorella ellipsoidea, Chlorella pyrenoidosa, Chlorella vulgaris, Dunaliella salina, Spirulina maxima, Spirulina platensis, Scenedesmus obliquus, Synechococcus sp, Navicula incerta* (Ejike et al., 2017). The schematic diagram for the recovery of peptides from marine algae is presented in Fig. 8.

The proteins and other components from the microalgae are released from the cells either by proteolytic enzymes (cellulase) or by sonication, the by-products (especially lipids) are removed by extraction and the proteins are converted to peptides

using proteolytic microorganisms or purified enzymes. Processing of the peptides is done by fractionation and purification techniques, particularly membrane ultrafiltration and chromatographic techniques using ion-exchange, affinity, and gel-permeation platforms, whose choice depends on the peptide structure, but also yield and feasibility for industrial scale-up (Samarakoon and Jeon, 2012).

An algal peptide: taurine (used in beverages, foods and nutritional supplements) with several functional and biological applications, has been separated from *Ulva pertusa* (Bellou et al., 2014).

Ko et al. (2012) investigated the peptic hydrolysis (using Protamex, Kojizyme, Neutrase, Flavourzyme, Alcalase, trypsin, α-chymotrypsin, pepsin and papain) of *Chlorella ellipsiodea* obtaining a pentapeptide (Leu-Asn-Gly-Asp-Val-Trp) with scavenging antioxidant activities in vitro, suggesting that *C. ellipsoidea* microalgae could become an attractive raw material for antihypertensive nutraceutical ingredients.

Kose and Oncel (2015) have analysed the effect of environmental conditions and nutritional mode on the biochemical composition of *Chlorella vulgaris SAG 211-12* cells in photomixotrophic cultivation, using a continuous 2L stirred tank photobioreactor in the scope of protein synthesis used as supplemental nutrition for cystic fibrosis patients. The authors used a modified BG11 culture medium, different organic carbon sources: glucose, sucrose, fructose, glycerol and xylose, and organic nitrogen sources such as yeast extract, peptone and urea. The results showed that carbon sources did not have a significant effect on protein synthesis, unlike nitrogen source: urea inhibited cell growth and the yeast extract and peptone increased the quantity of protein accumulated in the microalgae. The enzymatic hydrolysis was realised with pancreatin (mixture of amylases, lipases and proteases) and hydrolysis yielded over 50 %.

Functional foods containing microalgae-derived peptides have the potential to ameliorate cardiovascular disease, but in order to be largely used more studies that could prove the beneficial health, are required and also the development of feasible technologies need to be addressed (Ejike et al., 2017).

2.5. Lipids

Microalgae are excellent sources of lipids (up to 50 – 75 % of dry matter), making them useful organisms as source of long chain fatty acids, (used for the treatment of various diseases, including thrombosis, arthritis, atherosclerosis, and a variety of cancers) or as feedstock for biodiesel production. Lipids, especially ω-3 and ω-6 fatty acids (long-chain ω-3 and ω-6 fatty acids, like arachidonic acid, docosahexaenoic acid, eicosapentaenoic acid, gamma-linolenic acid) that cannot be synthesized by humans, but necessarily especially for babies, are produced by: *Arthrospira platensis, Crypthecodinium cohnii, Chaetoceros constrictus, Gloeobacter violaceus, Isochrysis galbana, Odontella sp., Oscillatoria agardhii, Tetraselmis viridis and Porphyridium cruentum* (Raposso at al., 2016).

Several microalgae species are able to accumulate appreciable (between 20 and 50 %) lipids quantities: *Porphyridium, Dunaliella, Isochrysis, Nannochloropsis, Tetraselmis, Phaeodactylum, Chlorella* and *Schizochytrium*. The schematic diagram for the recovery of lipids from marine algae is presented in Fig. 9.

The two main systems that can be used for microalgae growth are both applicable for lipids: for biodiesel production, the most appropriate would be open ponds, due to a low capital investment and operating cost of these systems, while for the production of polyunsaturated fatty acids closed photobioreactors are recommended (the higher cost can be covered by the high price of the products). As for the other presented compounds, the accumulation of lipids is increased under specific environmental stress conditions like nitrogen or phosphate limitation, high salinity, high temperature (Bellou et al., 2014; D'Alessandro and Filho, 2016). A number of processes can be applied for the harvest of biomass (depending on cell size and density, characteristics of the interest product), including those based on chemical, mechanical, physical, and biological methods, but for biodiesel production filtration, the combination of two processes seems to be preferred in order to reduce the cost of harvesting large quantities of algal biomass.

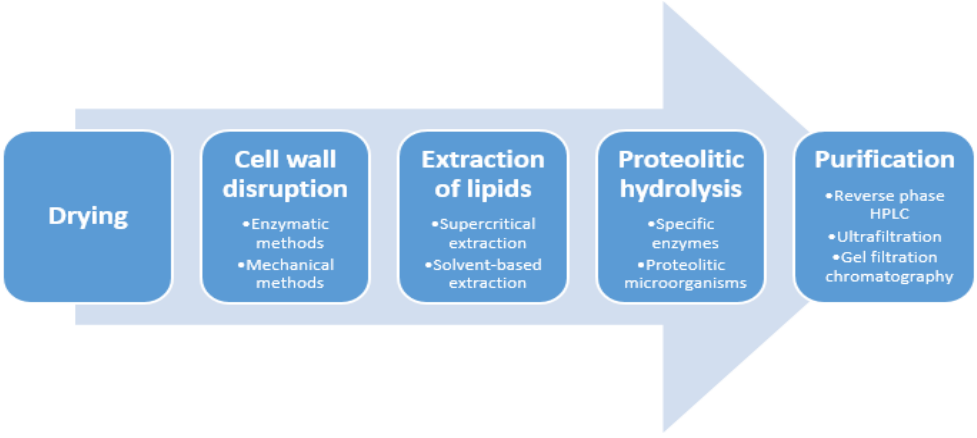


Fig. 8. Process diagram of bioactive peptides production

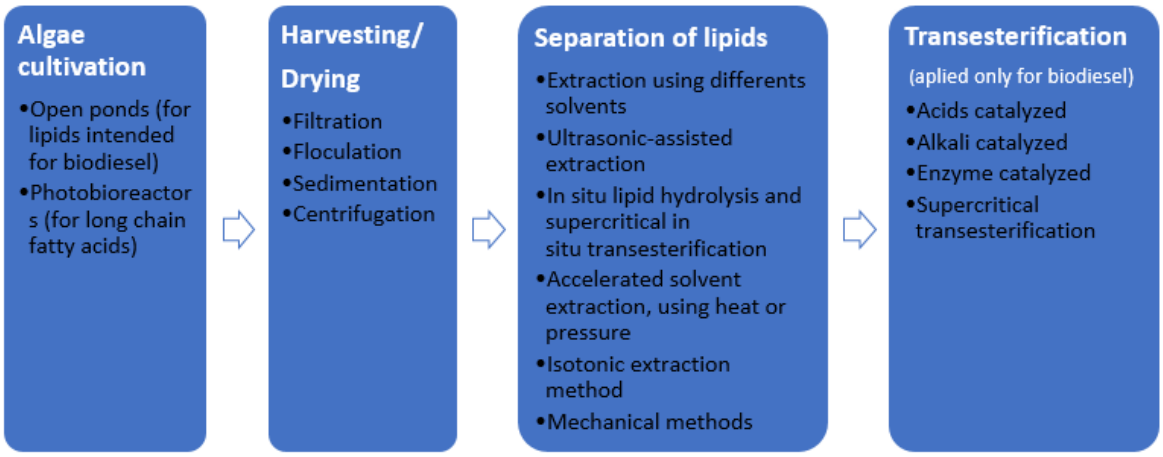


Fig. 9. Process diagram of polyunsaturated fatty acids and biodiesel production

For the extraction of lipids several techniques can be applied (some requires removing water from the biomass prior to lipid extraction for optimum results): organic solvents extraction with n-hexane, chloroform–methanol, methyl tert-butyl ether, 2-ethoxyethanol, electroporation, ultrasonic, and supercritical CO_2 methods (Kumar et al., 2015).

Biodiesel production from the lipids extracted is performed via a chemical conversion process known as transesterification, catalysed by acids, alkali or by enzymes. Recently an in-situ transesterification process with a strong catalyst dissolved in alcohol has been developed that does not require the lipids extraction from the biomass, but need to use dried microalgae (Du et al., 2016). Huang et al. (2016) investigated a thermochemical process including pyrolysis and hydrothermal liquefaction to transform all the organic components of wet microalgae rich in proteins: *Cyanobacteria sp.* and *Bacillariophyta sp.*, into oils with a yield of 21.1% d.w. and 18.2 respectively, at 325°C and 45 – 60 minutes. Moon et al. (2014) used a culture medium based on wastewater from a sugar factory (20 %) and hydrolysate of lipid extracted algal biomass (50 %) for the cultivation of *Ettlia sp.*, and obtained increased lipid productivity (from 5.8 to 95.5 mg/L/d). Du et al. (2017) analyzed the influence of freeze drying and cell breaking on lipids extraction efficiency from *Neochlorisoleo abundans*, proving that drying and cell breaking are not necessary for extraction with N-ethyl butylamine.

The biodiesel process can be more economical by combining lipid production with other applications: separation of carotenoids or polysaccharides, or an integration of wastewater as a medium for microalgal cultivation (Cheah et al., 2016). Millao and Uquiche (2016) studied lipids and carotenoids extraction from microalgae *Nannochloropsis gaditana* pelletized by supercritical CO_2, for the following conditions: 64°C and 956 kg/m^3 CO_2 density, obtaining a maximum yield of 152.9 g/kg d.w. for lipids and 773.7 mg/kg d.w. for carotenoids.

Although microalgae biodiesel production presents many advantages and it is well studied, due to high cost (especially for dewatering the biomass after harvest) there are no large scale algal biodiesel production facilities (Bellou et al., 2014). In order to reduce the cost for the process many efforts have been made for increasing by genetic mutations the lipids accumulation in the microalgae cell (more than 50 %), or to develop different separations methods: in situ lipid hydrolysis and supercritical in situ transesterification applied for wet microalgae (Levine et al., 2010;Valverde et al., 2016).

3. Conclusions

The technological importance of microalgae is clearly demonstrated by the multitude of high value compounds with wide applications in different areas: food additives, health food, cosmetic, pharmaceutical and medicine. The microalgal market is in continous growth: *Chlorella* and *Spirulina sp.* were the main algae grown industrially for human use (as nutritional supplement), but now *Dunaliella salina* is used to obtain beta-caroten, (1 million $/ton), or *Haematococcus pluvialis* for producing astaxanthin (10 millions $/ton). Carotenoids are the most important molecules extracted from microalgae, and even if a high productivity can be achieved due to optimized growth conditions and very efficient photobioreactors, a great interest still remains on the downstream part of the process.

High expectations are associated with the microalgae biotechnological field, and for this a number of challenges will need to be solved in the near future: optimization both of the production step (using **genetic mutation** to increase the content in a specific compound, **solid understanding** of intracellular activitiesand **process design** for industrial scale that allows the use of all by-products for sustainable and economically feasible production) and the downstream processes with minimal energy consumption, for the development of efficient processes for expansion at commercially scale.

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