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Research review paper

A review of high value-added molecules production by microalgae in light of the classification

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ABSTRACT

This work reviews applications of high added value molecules produced from microalgae. Older forms of valorization - health food and quality feed, polyunsaturated fatty acids, pigments, carbohydrates - are currently penetrating their markets. They are driven by desirable properties: texturer and dye for food industry, antioxidant for cosmetics and the appetite of the general public for biosourced compounds. Most recent developments, such as peptides, vitamins, polyphenols, phytosterols and phytohormones, are struggling to meet their market and reach economical competitiveness. Still they are pushed forward by the very powerful driver that is pharmaceutical industry. In addition this work also proposes to link microalgae phyla and related potential applications. This is done through highlighting of which bioactive compounds can be found in which phyla. While some seem to be restricted to aquaculture, Cyanobacteria, Chlorophyta and Rhodophyta show great promises.

1. Introduction

Algae are a group of organisms that live almost every habitat. While, most part of them are preferentially found in aquatic environments, they can also colonise other spaces including deserts, volcanic waters, highly acidic and frozen soils (Lim and Schenk, 2017). They form the basic component of food chains in the world's ecosystems. In addition, they are the main producers of oxygen and contribute to about 40% of global photosynthesis (Andersen, 2015; Hamed, 2016; Sharma and Rai, 2011).

Algae bring together macroalgae, commonly referred to as seaweed which are macroscopic and multicellular organisms, and microalgae, which are microscopic organisms commonly found in marine and freshwater. The later are the focus of this review. Microalgae have been used by man since the dawn of time. It started more than thousand years ago serving as food for indigenous populations. Still, the production of microalgal biomass really began to develop in the middle of the last century, starting first with Germany when post-war societies were apprehensive at a strong demographic growth and its implication to food security (Milledge, 2011; Spolaore et al., 2006). However, with the need for sustainable development, microalgae have really sparked academics and engineers attention over the last half-century for numerous reasons. First of all, as photosynthetic organisms, they can derive their energy from light and carbon from inorganic sources.

Furthermore, compared to higher plants, microalgae show higher annual photon-to-biomass conversion efficiencies (about 3% vs. < 1% for higher plants), and no intrinsic sensibility to seasonality (Blankenship et al., 2011; Perin et al., 2019). This feature combined with the great diversity of strains allow valuable molecules such as proteins, lipids, carbohydrates, pigments to be produced with high yields. Second, they can be grown on wastewater or non-agricultural land without pesticides hence not compromising the production of food or others products from crops. Furthermore, they are able to recycle atmospheric carbon dioxide and thus minimising associated environmental impacts. As a result, and driven by current energy, environmental and food challenges (Christaki et al., 2011; Hamed, 2016; Milledge, 2011), microalgal biotechnology steadily rose into power. Nowadays it can be split into four major research fields that are wastewater treatment, CO₂ sequestration, biofuel production and high-added value molecules production.

By having a look at scientific literature, it is safe to say that the first three fields have already been well explored and reviewed over the former decades. Fig. 1 presents the publication trends of those scientific communities. As one can see, very recently, the focus of microalgae applications has changed and is now mainly on the production of high value-added molecules rather than environmental applications. This recent shift in interest is probably related to the high cost of microalgae production even on a large scale. Among the main limitations, the low biomass concentration (about 3 g/L for autotrophic cultures) of

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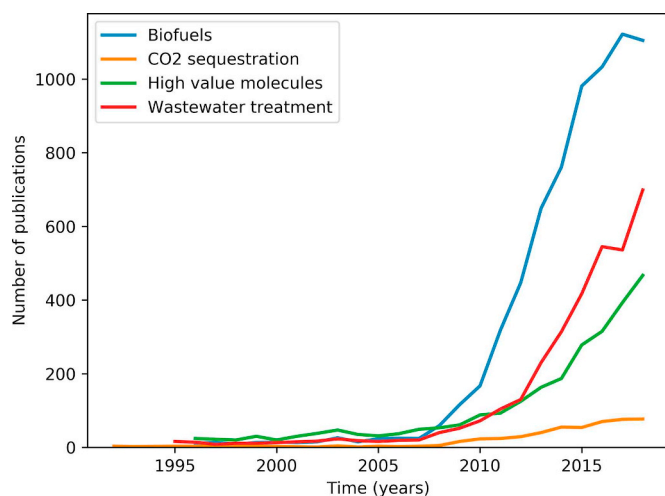


Fig. 1. Number of publications by category of microalgae applications over time. Results obtained on November 82,019, on the Web of Science search engine over a period until 2018 with the following keywords: TS = (microalgae AND (molecules OR bioactive OR compound)) for high-value molecules, TS = (microalgae AND ((CO2 OR Carbon) AND (sequestration OR mitigation))) for CO₂ sequestration, TS = (microalgae AND (wastewater OR “waste water” OR remediation OR polluted)) for wastewater treatment and TS = (microalgae AND (fuel OR biofuel OR oil OR biooil)) for biofuels.

microalgae cultures has a significant impact on the downstream processing. In particular, additional costs are required at the harvesting stage to successfully concentrate the cultures. These difficulties have led scientists to find new, higher added-value, strategies for valorisation of biomass. From this graph, we can see that the sequestration of CO₂ has a rather linear trend with a low slope suggesting a limited interest (linear fit since 2007, $R^2 = 0.977$). As for biofuels, the peak of research intensity seems to be passed (bell curve). Indeed, extensive research has already been led, and the conclusion is that commercial viability will be made possible only if high added value molecules are extracted from biomass beforehand (Rizwan et al., 2018). Finally wastewater treatment and high value-added molecules¹ clearly depict an exponential trend (exponential fit since 2007, $R^2 = 0.976$ and $R^2 = 0.991$ respectively). As a consequence, this review focuses on high-added value molecules production from microalgae and associated applications.

High added value molecules are a very board category. They range from lipids, proteins and carbohydrates with food and nutraceuticals applications to pigments and sterols with cosmetics and pharmaceutical aims. The broad range of applications comes from the fact that microalgae are one of the oldest life forms on Earth. They have evolved and adapted over billions of years, yielding a tremendous diversity and complexity. As classification can be of help when assessing the biotechnological potential of a strain, a reminder of a state of the art classification is the first section of this article. Then, for the sake of completeness and the reader interest, environmental applications are briefly mentioned and relevant contributions are pointed out. This is followed by the core of this paper, a review of high-added value molecules production and associated applications. Finally, a link is drawn between phylum and applications with the goal to ease strain selection for a specific application.

¹ It should be noted that for this last stream, broad keywords have been applied. However, unlike other applications, high value-added molecules from microalgae are very diverse and can have different applications. As a result, the volume of publications may have been underestimated with the use of very broad-spectrum keywords. With keywords more focused on the type of molecules such as “carbohydrate”, “protein” or else “phytosterol”, the trend remains the same but the volume of publication is larger than that presented in the graph.

2. Classification

Microalgae term ties together microscopic algae and oxygenic photosynthetic bacteria. Therefore the first distinction is between prokaryotes and eukaryotes (Stark and O’Gara, 2012). The key distinction between both types of cells is the presence of membrane-bound structures in eukaryotic cells which are lacking in prokaryotes. The latter were allegedly acquired by eukaryotes from evolution through endosymbiosis (Pulz and Gross, 2004; Rockwell et al., 2014). As a result, eukaryotes are larger, more complex, and can be either unicellular or multicellular while prokaryotes are simple and small single-celled organisms.

Traditionally, microalgae have been classified according to their photosynthetic pigments. Yet the current systems of classification take account of others criteria, among them cytological and morphological characters, cell wall constituents and chemical nature of storage products. Based on these features, some methods are commonly employed to identify and classify algal species including morphological observations under a microscope, molecular-based classification using specific short gene sequences (Groendahl et al., 2017) or more recently semi-automated or fully automated classification using a flow cytometer combined with computational methods (Correa et al., 2017; Drews et al., 2013; Sosik and Olson, 2007). Regardless of the approaches used to identify algal species, the classification system has changed many times over the years. Currently, there is no consensus among the taxonomists around the world to use one classification over another (Heimann and Huerlimann, 2015; Malcata et al., 2018). However, one of the latest classification models includes two main domains, Prokaryota and Eukaryota gathering seven kingdom: Archaeobacteria, Eubacteria, Protozoa, Chromista, Fungi, Plantae and Animalia (Ruggiero et al., 2015).

Keeping in mind that the taxonomic organization may change as information accumulates, the current microalgal classification considers eight major phyla belonging to four of the seven kingdoms depicted in Fig. 2. While a large majority of microalgae are nested in the Eukaryota domain and distributed in seven main phyla, there is only one representative phylum of microalgae in the Prokaryota domain grouped under the name Cyanobacteria. Despite the low representation in this domain, Cyanobacteria are among the most abundant phylum alongside Chlorophyta and Heterokontophyta phyla (García et al., 2017; Singh and Saxena, 2015; Venkatesan and Manivasagan, 2015)

3. Applications

3.1. Summary of environmental applications

Owing to the increasing deterioration of our environment and the need for energy, research have been focused on recycling and resource recovery. Within this context, microalgae have been studied as an appropriate response to the current environmental issues due to potential benefits they offer (Quinn and Davis, 2015; Shilton and Guieysse, 2010; Venkatesan and Manivasagan, 2015; Ward et al., 2014), namely:

- Their ability to integrate carbon from combustion gases as a source of carbon (Ho et al., 2011; Milledge, 2011; Razzak et al., 2013; Shilton and Guieysse, 2010; Sierra et al., 2017),
- Their potential of growing on wastewater and incorporating pollutants into their metabolism as nutrients (Abinandan and Shanthakumar, 2015; Goncalves et al., 2017; Muñoz and Guieysse, 2006; Sierra et al., 2017),
- The potential of their macronutrients composition (high carbohydrates and proteins) for bioplastics production (Hempel et al., 2011; Rahman and Miller, 2017; Zeller et al., 2013; Zhang et al., 1999; Zhang et al., 2000).
- Their use as biofertilizer owing to their rich micronutrients profile

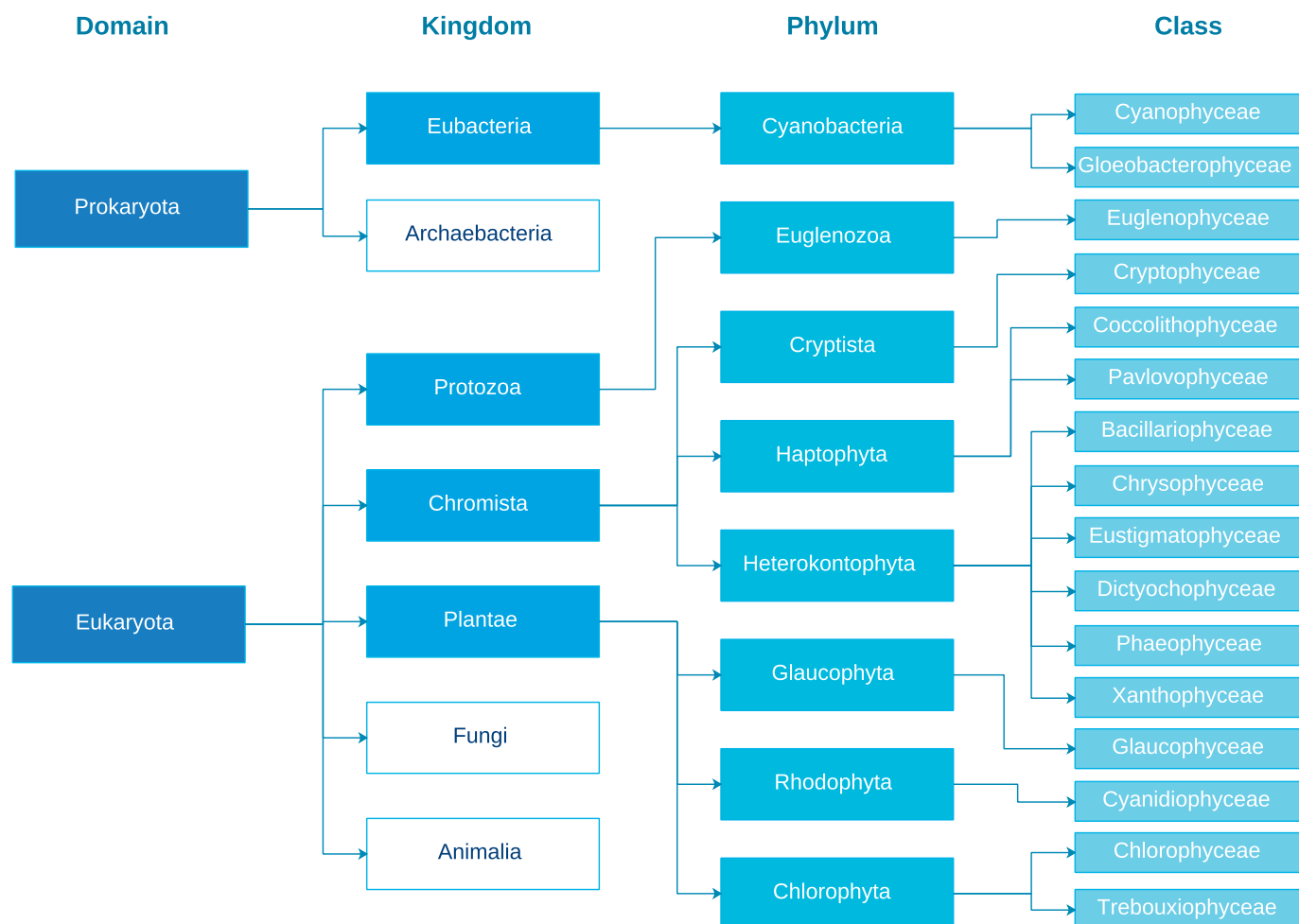


Fig. 2. Major microalgae phyla distribution as per seven-kingdom classification scheme inspired from (Ruggiero et al., 2015) with classes used for various biotechnological applications.

(nitrogen, potassium and phosphorus (Borowitzka, 1995; Hamed, 2016; Priyadarshani and Rath, 2012; Pulz and Gross, 2004; Siqueira et al., 2018)),

Microalgae have also sparked a major wave of research on the possibility of turning them into biofuels summarized in the Fig. 3. Nevertheless, despite the high potential of microalgae, the development of an efficient microalgae-based biofuel production chain remains a major challenge for commercial deployment, both technically and economically (Chiaromonti et al., 2017; Khanra et al., 2018; Milledge, 2011; Valverde et al., 2016; Wijffels, 2008).

3.2. Health food and quality feed

Thanks to their composition as well as their simplicity of cultivation, microalgae garnered interest over time as a solution to feed undernourished populations in the context of malnutrition in developing countries (Milledge, 2011). Furthermore, in recent years, consumer concerns regarding health and safety issues related to the consumption of processed foods have increased (Vaz et al., 2016). In this respect, microalgae would appear to be potential actors in the trend of using natural additives and are expected to play an important role in the food industry, in particular as nutraceuticals (Bishop and Zubeck, 2012; García et al., 2017; Hamed, 2016; Milledge, 2011; Spolaore et al., 2006). As a result, many companies have started to market functional foods containing microalgal biomass. This latter can either be directly marketed under different forms such as tablets, capsules and liquids or

integrated into products and act as a natural nutritional supplement or as a natural rheology improver product (Hamed, 2016; Pulz and Gross, 2004).

Currently, the market is dominated by four genera of microalgae (Brennan and Owende, 2010; Hamed, 2016; Spolaore et al., 2006; Vaz et al., 2016) which are *Arthrospira* with more than 12,000 tons of biomass produced every year, followed by *Chlorella* with about 5000 tons per year. *Dunaliella salina*, mainly marketed for its β -carotene, has an annual production of 3000 tons and finally *Aphanizomenon* is produced at a level of 1500 tons per year. Health food production systems also cultivate to a lesser extent the green alga *Scenedesmus* (Milledge, 2011; Pulz and Gross, 2004). It should be underlined that these species benefit from the GRAS status, *i.e.* Generally Regarded As Safe given by FDA for food purposes and are thus considered as safe and valuable for human consumption (de la Noue and de Pauw, 1988; García et al., 2017; Hamed, 2016; Spolaore et al., 2006).

In addition to its use in human food, microalgae have shown a growing interest in animal nutrition as feed additive. Microalgal biomass can be incorporated into the diet of a wide variety of animals ranging from fish to pets and livestock (Hamed, 2016; Priyadarshani and Rath, 2012; Pulz and Gross, 2004; Spolaore et al., 2006; Yaakob et al., 2014). They are mainly used to improve the physiology of animals but also the organoleptic properties of the final consumer product (Hamed, 2016). Currently, about 30% of current global microalgae production is sold to the feed industries (Hamed, 2016; Milledge, 2011; Raja et al., 2008; Spolaore et al., 2006).

The perspectives of the use of microalgae as food and feed lies in the

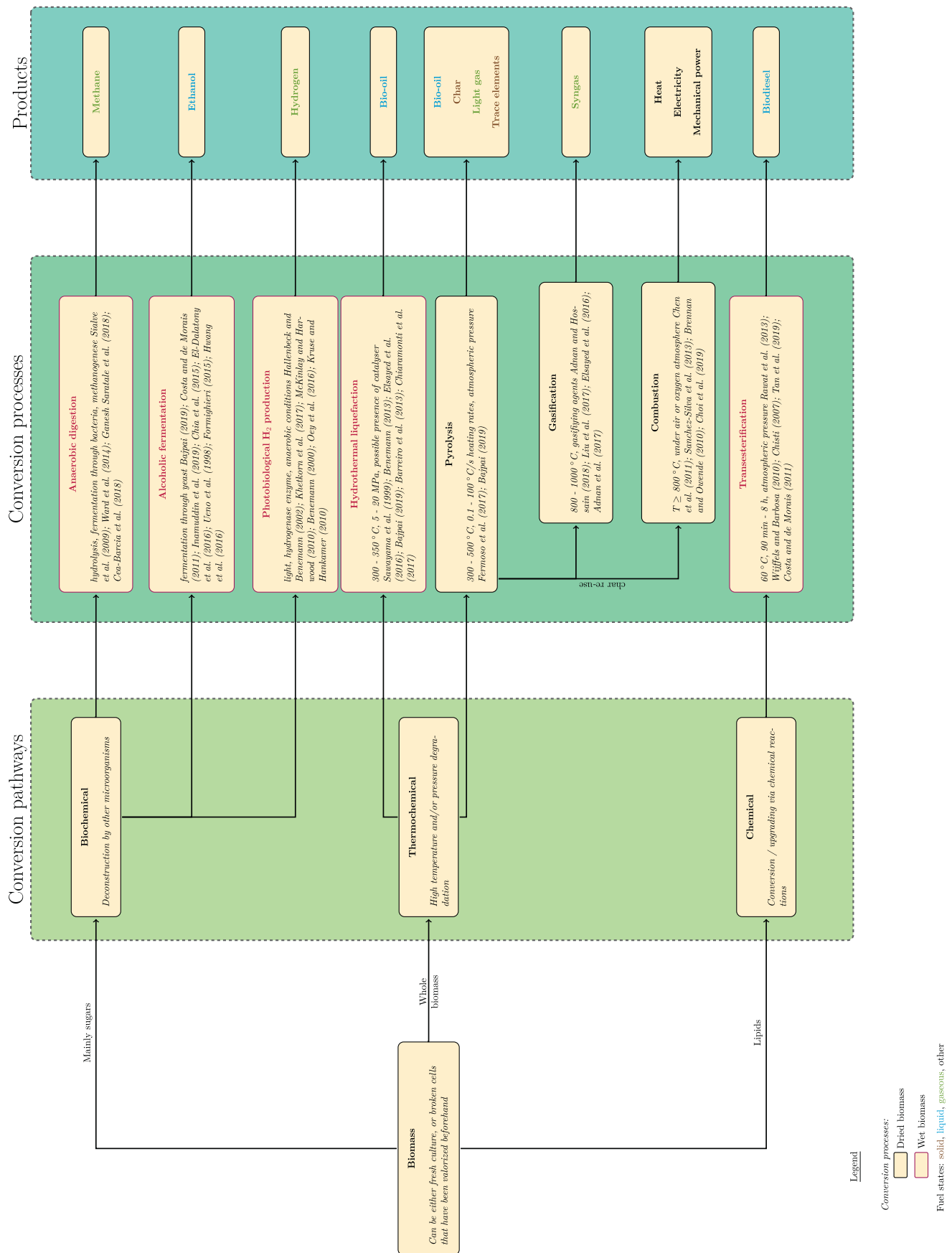


Fig. 3. Main bioactive compounds expressed in microalgae in relation to their classification and possible applications as high value added products. Numbers of species obtained from (Guiry, 2012; Guiry and Guiry, 2020). Blue circle: pharmaceuticals; pink circle: cosmetics; yellow circle: nutraceuticals and green circle: food. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Biochemical composition of different microalgae with controlled growth conditions. Results expressed as % of dry matter.

Strains	Original study parameters	Biochemical composition (% dry matter)			References
		Proteins	Carbohydrates	Lipids	
<i>Arthrospira maxima</i>	– Lighting conditions: natural sunlight	56–62	18–23	13	(Torzillo et al., 1986)
<i>Arthrospira maxima</i>	– Lighting conditions: 180 $\mu\text{molphotons}/\text{m}^2/\text{s}$ – Temperature: from 20 to 40 °C	63–70	10–20	6–7	(Oliveira et al., 1999)
<i>Arthrospira platensis</i>	– Lighting conditions: 180 $\mu\text{molphotons}/\text{m}^2/\text{s}$ – Temperature: from 20 to 40 °C	59–72	11–20	6–7	(Oliveira et al., 1999)
<i>Arthrospira platensis K-2</i>	– Lighting conditions: 30 $\mu\text{molphotons}/\text{m}^2/\text{s}$ – Photoperiod: 12:12 h (L/D cycles)	38–53	13–25	6–15	(Madkour et al., 2012)
<i>Arthrospira platensis LEB-52</i>	– Nitrogen sources: ammonium nitrate or urea – Lighting conditions: 31.35 $\mu\text{molphotons}/\text{m}^2/\text{s}$ – Photoperiod: 12:12 h (L/D cycles) – Temperature: 30 and 35 °C	57–70	–	7–10	(Colyer et al., 2005)
<i>Chlamydomonas reinhardtii</i>	– Nitrogen source: sodium nitrate – Lighting conditions: continuous illumination	65	23	–	(Mahdy et al., 2014)
<i>Chlamydomonas reinhardtii CC-400 cw15 mt+</i>	– Lighting conditions: continuous illumination of 80 $\mu\text{molphotons}/\text{m}^2/\text{s}$ – Nitrogen source: ammonium chloride	45	25	9	(Park et al., 2015)
<i>Chlorella vulgaris</i>	– Lighting conditions: 150 $\mu\text{molphotons}/\text{m}^2/\text{s}$ – Photoperiod: 16:8 h (L/D cycles)	12–45	8–35	3–6	(Chia et al., 2013)
<i>Chlorella vulgaris</i>	– Lighting conditions: continuous illumination of 60 $\mu\text{molphotons}/\text{m}^2/\text{s}$ – Nitrogen source: potassium nitrate	7	63	29	(Lv et al., 2010)
<i>Chlorella vulgaris</i>	– Lighting conditions: daylight illumination	51	18	7	(Morris et al., 2008)
<i>Chlorella vulgaris ESP-6</i>	– Lighting conditions: 60 $\mu\text{molphotons}/\text{m}^2/\text{s}$ – Temperature: 28 °C	48	18	13	(Ho et al., 2013)
<i>Chlorella vulgaris FSP-E</i>	– Lighting conditions: 60 $\mu\text{molphotons}/\text{m}^2/\text{s}$ – Temperature: 28 °C	60	12	12	(Ho et al., 2013)
<i>Dunaliella salina</i>	Nothing specific	26	40	18	(Muhaemin and Kaswadji, 2010)
<i>Dunaliella tertiolecta</i>	– Lighting conditions: 70–80 $\mu\text{molphotons}/\text{m}^2/\text{s}$ – Photoperiod: 12:12 h (L/D cycles)	20	12	15	(Brown, 1991)
<i>Isochrysis galbana</i>	– Photoperiod: 12:12 h (L/D cycles)	16–22	3–6	4–5	(Gnouma et al., 2017)
<i>Isochrysis galbana</i>	The strain was received freeze-dried and was analysed without further processing	42	10	25	(Bonfanti et al., 2018)
<i>Isochrysis galbana</i>	– Lighting conditions: 1000 lx – Temperature 20 °C	29–42	4–10	20–24	(Whyte, 1987)
<i>Isochrysis galbana</i>	– Lighting conditions: 70–80 $\mu\text{molphotons}/\text{m}^2/\text{s}$ – Photoperiod: 12:12 h (L/D cycles)	29	13	23	(Brown, 1991)
<i>Isochrysis galbana TK1</i>	– Temperatures: 15 °C and 30 °C – Lighting conditions: 200 $\mu\text{molphotons}/\text{m}^2/\text{s}$	27–32	25–33	18–25	(Zhu et al., 1997)
<i>Isochrysis sp.</i>	– Lighting conditions: 80 $\mu\text{molphotons}/\text{m}^2/\text{s}$ – Salinity 25‰	30	16	23	(Renaud et al., 1999)
<i>Nannochloropsis oculata</i>	– Lighting conditions: 350 $\mu\text{mol photons}/\text{m}^2/\text{s}$ – Photoperiod: 12:12 h (L/D cycles) – Salinity: 33‰	26	29	26	(Paes et al., 2016)
<i>Nannochloropsis oculata</i>	– Lighting conditions: 80 $\mu\text{molphotons}/\text{m}^2/\text{s}$ – Photoperiod: 12:12 h (L/D cycles) – Temperature: 26 °C - [FeCl ₃]: 0,15 to 63 mg/L	–	–	21–33	(Sabzi et al., 2018)
<i>Nannochloropsis oculata</i>	– Lighting conditions: 70–80 $\mu\text{molphotons}/\text{m}^2/\text{s}$ – Photoperiod: 12:12 h (L/D cycles) – Temperature: 20 °C	35	7,8	18	(Brown, 1991)
<i>Nannochloropsis sp.</i>	– Lighting conditions: different light spectra - Photon flux density < 100 $\mu\text{molphotons}/\text{m}^2/\text{s}$	20–25	18–30	44–60	(Vadiveloo et al., 2015)
<i>Pavlova salina</i>	– Lighting conditions: 70–80 $\mu\text{molphotons}/\text{m}^2/\text{s}$ – Photoperiod: 12:12 h (L/D cycles)	26	7	12	(Brown, 1991)
<i>Phaeodactylum tricorutum</i>	– PBR and circular pond comparison – Lighting conditions: daylight	30–59	9–20	24–36	(Silva Benavides et al., 2013)
<i>Phaeodactylum tricorutum</i>	– Lighting conditions: 18–72 $\mu\text{molphotons}/\text{m}^2/\text{s}$	38–51	18–28	9–30	(KaiXian and Borowitzka, 1993)
<i>Phaeodactylum tricorutum</i>	– Lighting conditions: 70–80 $\mu\text{molphotons}/\text{m}^2/\text{s}$ – Photoperiod: 12:12 h (L/D cycles)	30	8	14	(Brown, 1991)
<i>Phaeodactylum tricorutum</i>	– [Nitrogen]: 0.88 mM	32	19	29	(KaiXian and Borowitzka, 1993)
<i>Porphyridium cruentum</i>	– Lighting conditions: 50 and 200 $\mu\text{molphotons}/\text{m}^2/\text{s}$ – Salinity: 33‰ – Nitrogen sources: ammonium and nitrate	5–10	25–37	8–18	(Sánchez-Saavedra et al., 2018)
<i>Porphyridium cruentum 161</i>	– Lighting conditions: 60 $\mu\text{molphotons}/\text{m}^2/\text{s}$	–	30	10	(Hong et al., 2017)
<i>Porphyridium cruentum 161</i>	– Mode: continuous mode (dilution rate 10 h) – Lighting conditions: daylight	34	32	7	(Reboloso Fuentes et al., 2000)
<i>Scenedesmus obliquus CNW-N</i>	– Lighting conditions: 420 $\mu\text{molphotons}/\text{m}^2/\text{s}$ – Temperature: 28 °C	15–41	38–52	12–22	(Ho et al., 2012)
<i>Tetraselmis sp.</i>	– Lighting conditions: 80 $\mu\text{molphotons}/\text{m}^2/\text{s}$ – Salinity: 25‰	13–14	8–9	26–30	(Renaud et al., 1999)
<i>Tetraselmis suecica</i>	– Lighting conditions: 1000 lx	33–34	8–10	6–7	(Whyte, 1987)
<i>Tetraselmis suecica</i>	– Lighting conditions: 70–80 $\mu\text{molphotons}/\text{m}^2/\text{s}$ – Photoperiod: 12:12 h (L/D cycles)	31	12	10	(Brown, 1991)

Table 2

General composition of different microalgae under stressful conditions: light stress (above 200 $\mu\text{molphotons}/\text{m}^2/\text{s}$); nitrogen stress (exhausted nitrate source); combined light and nitrogen stresses; and chemical stress.

Strains	Original study parameters	Type of stress	Biochemical composition (% dry matter)			References
			Proteins	Carbohydrates	Lipids	
<i>Chlamydomonas reinhardtii</i> CC-400 cw15 mt+	– Lighting conditions: continuous illumination of 80 $\mu\text{molphotons}/\text{m}^2/\text{s}$ – Nitrogen starvation	Nitrogen	36	17	41	(Park et al., 2015)
<i>Chlamydomonas reinhardtii</i> UTEX 90	– Mode: fed-batch culture for 96 h – Lighting conditions: continuous illumination of 450 $\mu\text{molphotons}/\text{m}^2/\text{s}$	Light	9	60	–	(Choi et al., 2010)
<i>Chlorella vulgaris</i>	– Lighting conditions: 150 $\mu\text{molphotons}/\text{m}^2/\text{s}$ – Photoperiod: 16:8 h (L/D cycle) – Nitrogen limitation – Cadmium stress	Nitrogen	10–25	10–59	5–16	(Chia et al., 2015)
<i>Chlorella vulgaris</i> ESP-6	– Lighting conditions: 60 $\mu\text{molphotons}/\text{m}^2/\text{s}$ – Temperature: 28 °C – Nitrogen starvation	Nitrogen	23	49	15	(Ho et al., 2012)
<i>Chlorella vulgaris</i> FSP-E	– Lighting conditions: 60 $\mu\text{molphotons}/\text{m}^2/\text{s}$ – Temperature: 28 °C – Nitrogen starvation	Nitrogen	21	54	19	(Ho et al., 2012)
<i>Isochrysis galbana</i>	– Lighting conditions: continuous illumination of 150 $\mu\text{molphotons}/\text{m}^2/\text{s}$ – Temperature: 27 °C – Nitrogen starvation	Nitrogen	12–30	–	24–47	(Kaplan et al., 1986)
<i>Nannochloropsis oculata</i>	– Lighting conditions: 350 $\mu\text{molphotons}/\text{m}^2/\text{s}$ – Photoperiod: 12:12 h (L/D cycles) – Salinity: 33‰ – Nitrogen starvation	Light and nitrogen	18	29	34	(Paes et al., 2016)
<i>Nannochloropsis</i> sp.	– Mode: semicontinuous regime - Lighting conditions: from 0 to 480 $\mu\text{molphotons}/\text{m}^2/\text{s}$ – Photoperiod: 12:12 h (L/D cycles)	Light	10–43	20–29	33–61	(Fábregas et al., 2004)
<i>Pavlova</i> sp.	– Lighting conditions: 180–220 $\mu\text{molphotons}/\text{m}^2/\text{s}$ – Salinity: 34–35‰	Light	34–54	30–44	16–29	(Ponis et al., 2006)
<i>Phaeodactylum tricorutum</i>	– Nitrogen limited and nitrogen free media	Nitrogen	16–25	15–17	32–41	(KaiXian and Borowitzka, 1993)
<i>Porphyridium cruentum</i> 161	– Lighting conditions: 60 $\mu\text{molphotons}/\text{m}^2/\text{s}$ – Nitrogen limited	Nitrogen	–	43	10	(Hong et al., 2017)
<i>Porphyridium cruentum</i> VISCHER 1935/107	– Lighting conditions: 8000 lx	Light	27–38	40–57	9–12	(Vasileva and Ivanova, 2019)
<i>Scenedesmus obliquus</i>	– Lighting conditions: continuous illumination from 10 to 1000 $\mu\text{mol}/\text{m}^2/\text{s}$ – Pulsed light at 1500 $\mu\text{mol}/\text{m}^2/\text{s}$ and 10 Hz – NaCl stress	Light	30–40	17–40	35–50	(Gris et al., 2014)
<i>Scenedesmus obliquus</i> HM103382	– Continuous light of 40 $\mu\text{molphotons}/\text{m}^2/\text{s}$ – Temperature: 27 °C	Chemical	–	–	18–34	(Salama et al., 2013)
<i>Scenedesmus</i> sp. CCNM 1077	– Photoperiod: 12:12 h (L/D cycles) – Nitrogen limitation and starvation	Nitrogen	17–50	16–45	16–29	(Pancha et al., 2014)
<i>Tetraselmis</i> sp.	– Continuous illumination at 100–120 $\mu\text{molphotons}/\text{m}^2/\text{s}$ at both sides of PBR – Different sources of nitrogen	Nitrogen	22–48	17–42	16–28	(Kim et al., 2016)
<i>Tetraselmis</i> sp.	– Continuous illumination at 100–120 $\mu\text{molphotons}/\text{m}^2/\text{s}$ at both sides of PBR – Nitrogen limitation	Nitrogen	17–22	42–55	13–16	(Kim et al., 2016)

diversity of biomass composition. This can be achieved either by strain selection (Table 1) or growth condition manipulation (Table 2). Indeed, they are able to modulate their biochemical composition in response to a change in their environment. As a consequence, researchers have developed strategies based on metabolic imbalances which divert the electron energy towards the selected target (Gifuni et al., 2019). These are usually referred to as *stressing procedures* and feature nutrient depletion, high light irradiance, extreme pH, temperature, high salinity or metal concentration.

Beyond its macronutrient composition, microalgae are able to express and accumulate secondary metabolites under stressful conditions. This paves the way to targeted bioactive compounds production, and eventually penetrate cosmetics and pharmaceutical markets.

3.3. High-value molecules

High-value molecules term can be broken into several sub-categories

as it covers a very wide range of bioactive compounds. In this review, they are divided into: polyunsaturated fatty acids (PUFAs), pigments, carbohydrates, peptides, vitamins, polyphenols, phytosterols and hormones. For the sake of readability, the applications of those family of molecules extracted from microalgae are synthetically presented in Table 3 with respect to four fields of applications: food, nutraceuticals, pharmaceuticals and cosmetics. More specific details about their productions and key activities are emphasized in the text hereinafter.

3.3.1. Polyunsaturated fatty acids (PUFAs)

There are two main groups of lipids in microalgae: the lipids produced by photosynthesis and stored in the cell known as storage lipids (mainly triglycerides) and lipids being an integral part of the cell structure known as structural lipids (phospholipids and sterols) (Hamed, 2016).

Fatty acids belong to the storage lipids. They represent one of the primary metabolites. Within them, polyunsaturated fatty acids (PUFAs)

Table 3
Main molecules of commercial interest extracted from microalgae in relation to their field of application.

Molecules	Applications	References
PUFAs	<i>Food:</i> - <i>Nutraceuticals:</i> Enrich formula in omega-3	(Adarme-Vega et al., 2012; Christaki et al., 2011; Doughman et al., 2007; Hamed, 2016; Milledge, 2011; Odjadjare et al., 2017; US EPA, 2015; Yaakob et al., 2014)
DHA, EPA	<i>Pharmaceuticals:</i> Positif on blood system (pressure, coagulation), act on the proper function and development of nervous and visual system, reduce occurrence of chronic diseases (diabete, artritis, cardiovascular disease and obesity), reduce the level of cholestrol triglycerids, prevent arthritis, Alzheimer's disease, psoriasis and certain type of cancer, anti-inflammatory properties <i>Cosmetics:</i> -	
Chlorophylls	<i>Food:</i> Green dyeing agents <i>Nutraceuticals:</i> Antioxidant properties	(Borowitzka, 1995; Odjadjare et al., 2017; Yaakob et al., 2014)
Chlorophyll a and b	<i>Pharmaceuticals:</i> Antioxidant properties, vitamin precursors, immune activator, anti-inflammatory properties, cancer prevention (colorectal), cytotoxic towards tumoral cells, stimulate liver function, increase bile secretion, increase hemoglobin, promote rapid cell growth <i>Cosmetics:</i> Dyeing agents, additive agents, antioxidant properties, mask odours	
Carotenoids	<i>Food:</i> Dyeing agents whose color depends on the molecule (red, orange, yellow) <i>Nutraceuticals:</i> Antioxidant properties	(; Berthon et al., 2017; Bhalamur et al., 2018; Borowitzka, 1995; García et al., 2017; Gouveia et al., 2010; Hamed, 2016; Mourelle et al., 2017; Odjadjare et al., 2017; Siqueira et al., 2018; Sousa et al., 2008; Yaakob et al., 2014)
β -caroten, Astaxanthin, Lutein, Lycopene, Violaxanthin	<i>Pharmaceuticals:</i> Antioxidant properties, vitamin precursors, immune activator, anti-inflammatory properties, antihypertensive properties, neuroprotective properties, protective activities against cancer, atherosclerosis, ulcers and carviocvascular diseases, prevent macular degeneration, reduce the prevalence of metabolic syndrome, adiposity and serum triglyceride concentrations, strengthen immune resistance to viral, bacterial, fungal and parasitic infections <i>Cosmetics:</i> Antioxidant properties, photoprotective properties, dyeing agents	
Phycobiliproteins	<i>Food:</i> Dyeing agents whose color depends on the molecule (red, blue, light blue, magenta) <i>Nutraceuticals:</i> -	(Bhalamur et al., 2018; Borowitzka, 1995; Gouveia et al., 2010; Hamed, 2016; Milledge, 2011; Odjadjare et al., 2017; Siqueira et al., 2018; Yaakob et al., 2014)
Phycocerythrin, Phycocyanin, Allophycocyanin, Phycocerythrocyanin	<i>Pharmaceuticals:</i> Fluorescent properties, markers of certain immunological methods, antioxidant properties, anti-inflammatory properties, neuroprotective properties, hepatoprotective properties <i>Cosmetics:</i> Dyeing agents	
Exopolysaccharides	<i>Food:</i> Water retention capacity, texturing agents, thickening agents, gelling agents, stabilizers, emulsifiers, shelf-life extenders <i>Nutraceuticals:</i> - <i>Pharmaceuticals:</i> Antioxidant properties, antiviral properties (HIV, Herpes simplex type 1), antitumor properties, anticancer properties, anticoagulant properties, antilipidemic properties, immune activator, anti-inflammatory properties, fight against certain diseases (atherosclerosis, cardiovascular disorders, HIV), thickening and gel-forming properties for drug design <i>Cosmetics:</i> Rheology modifiers, conditioners, anti-inflammatory properties, anti-redness properties, antimicrobial properties, antiviral properties, antioxidant properties, moisturizing agents, healing agents, emulsifiers, substitutes for hyaluronic acid, stimulate collagen synthesis, protective activities against enzymatic proteolysis	(Ahmed et al., 2014; de la Noue and de Pauw, 1988; Martins et al., 2014; Mourelle et al., 2017; Venugopal, 2016; Berthon et al., 2017)
Proteins	<i>Food:</i> Emulsifying agents, foaming agents, thickening agents, gelling agents <i>Nutraceuticals:</i> Hypolipidemic, hypoglycemic, anorectic, anorexigenic <i>Pharmaceuticals:</i> Anti-inflammatory properties, antihypertensive properties, anticancer properties, antibacterial properties, antioxidant properties, platform for recombinant proteins production <i>Cosmetics:</i> Stimulate collagen synthesis, reduce vascular imperfections, photoprotective properties, antioxidant properties	(Becker, 2007; Berthon et al., 2017; Christaki et al., 2011; Kim and Kang, 2011; Martins et al., 2014; Mourelle et al., 2017; Sadeghi et al., 2018; Sheih et al., 2009; Siqueira et al., 2018; Stolz, 2005; Vo et al., 2013; Yaakob et al., 2014); Khanra et al., 2018; Bernaerts et al., 2019; Gong et al., 2011; Dixon and Wilken, 2018)
Vitamins	<i>Food:</i> - <i>Nutraceuticals:</i> Precursor properties of some important enzyme cofactors <i>Pharmaceuticals:</i> Antioxidant properties <i>Cosmetics:</i> -	(Galasso et al., 2019)
Polyphenols	<i>Food:</i> - <i>Nutraceuticals:</i> - <i>Pharmaceuticals:</i> Antioxidant properties, anti-inflammatory properties, anti-cancer properties, anti-allergic properties, anti-diabetes properties, antimicrobial properties, antifungal properties, antimycotoxigenic properties, improve cardiovascular-associated disorders <i>Cosmetics:</i> -	(Galasso et al., 2019; Gómez-Guzmán et al., 2018; Li et al., 2007; Scaglioni et al., 2019)
Phytosterols	<i>Food:</i> - <i>Nutraceuticals:</i> - <i>Pharmaceuticals:</i> Reduce LDL-cholesterol, promote cardiovascular health, anti-inflammatory properties, anti-atherogenicity properties, anti-cancer properties, anti-oxidative properties, protection properties against nervous system disorders <i>Cosmetics:</i> -	(Ahmed et al., 2015; Kim et al., 2008; Kritchevsky and Chen, 2005; Woyengo et al., 2009)

are of note. They are biomolecules made of a long unsaturated hydrocarbon chain containing more than one double bonds with attractive properties for both food and pharmaceutical industries. From a medical point of view, PUFAs have been proven to have health benefits. Several studies highlighted the essential nature for the development of the human nervous system and visual abilities supported by the United States Environmental Protection Agency, the Food and Drug Administration as well as the Food and Agriculture Organization (Nations, 2010; Singh, 2005; US EPA, 2015). In addition, these fatty acids would also help to reduce the occurrence of various chronic diseases such as diabetes, arthritis, cardiovascular disease, and obesity (Hamed, 2016). Furthermore, a medical review based on both *in vitro* and *in vivo* studies in animals and humans on the ω -3 fatty acids supplementation underlined their anti-inflammatory, anti-proliferative and anticarcinogenic effects (Jho et al., 2004).

More specifically, ω -3 and ω -6 are essential for human being as they are not able to produce themselves these lipids which are necessary for their basal metabolism (Christaki et al., 2011; Doughman et al., 2007; Gouveia et al., 2010; Odjadjare et al., 2017). Therefore, they have to obtain them from an exogenous source. It has emerged that one of the promising sources of omega is microalgae, the primary producers of ω -3 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Accordingly, numerous studies focused on the production of PUFAs from microalgae.

In a very general way, microalgal fatty acids profile is relatively preserved within a phylum (Sahu et al., 2013) while their content varies within species according to environmental factors. Though, the ω -3 (EPA and DHA) content in microalgae is naturally relatively low. As an example, 12 strains of microalgae belonging to two distinct phyla were screened in a study (Sahu et al., 2013). Of all microalgae, only *Chlorella* had quantifiable levels of DHA and EPA (3.24 and 8.9% of total FAME respectively). Thus, EPA and DHA productivity improvement strategies within the cells have been implemented. The first and most well-known approach is to modify environmental parameters to affect internal metabolism. As a result, several studies have been focused on the impact of different environmental factor on the lipids productivity (Ramesh Kumar et al., 2019; Wang et al., 2019). One of the most employed strategy consists in partially or completely depriving microalgae of nitrogen to achieve higher lipid productivity. One of the most striking example is the growth of *Chlamydomonas reinhardtii* CC-400 *cw15 mt+*. Under normal conditions, the strain is able to express 9% dry matter of lipids while under nitrogen starvation it yields 41% dry matter (Park et al., 2015). Another way to stimulate lipid production is to act directly on the metabolism of the microalgae. It has been shown that under heterotrophic conditions, microalgae obtain higher lipid yields (Ramesh Kumar et al., 2019; Sun et al., 2018). As an example, after 3 days of cultivation, a *Thraustochytrid* strain 12B is able to express 57.8% lipids in biomass with 43.1% of DHA in it (Raghukumar, 2008). Besides, increasing experimental evidence seems to highlight that an appropriate level of intracellular Reactive Oxygen Species (ROS) can improve lipid accumulation although the metabolic mechanism is not well known yet (Sun et al., 2018). This is made possible by, among other things, the controlled addition of oxygen during cultivation.

However, these conditions, although favourable for lipid accumulation, are not so for microalgae growth leading to reduced growth rates. One way to overcome this problem is to use a two-phase approach. This strategy consists in first the cultivation of microalgae under sufficient nutrients conditions to stimulate higher growth rate and by extension higher biomass. These cells are then exposed to more severe conditions such as nutrient starvation for accumulation of the lipids. As an example, a study following this methodology was conducted on *Nannochloropsis oculata* cells (Paes et al., 2016). Under both control conditions, *i.e.* repleted nitrogen, and nitrogen limitation conditions, the final cell yields were similar. However, the cell volume increased under nitrogen limitation from 14.1 to 18.7 μm^3 suggesting a lipid accumulation inside the cells. This was confirmed by analyses. The

lipid content increased from 26 to 34% of dry matter. Another way to improve lipid yields without substantial loss of biomass is to combine this approach with strain breeding or genetic engineering approach (Ramesh Kumar et al., 2019; Wang et al., 2019).

Currently, the main source of EPA and DHA in food are fatty fish such as salmon. Yet PUFAs derived from this source have low oxidative stability, can contain toxins as well as releasing unpleasant smell and taste, thus limiting their application (Bhalamur et al., 2018; Odjadjare et al., 2017; Yaakob et al., 2014). Consequently, microalgae are increasingly emerging as an alternative to the use of fish oil (Ward and Singh, 2005). However, this alternative is limited to the production of DHA (Odjadjare et al., 2017). Indeed, until recently, the production of EPA rich algal oil has been restricted to laboratory scale (Milledge, 2011). As a result, DHA is currently the only product on the market (Yaakob et al., 2014). The most common microalgae used for its production are from the genera *Cryptocodinium*, *Schizochytrium* and *Ulkenia* found in the kingdom Chromista (Hamed, 2016; Winwood, 2013). These microalgae are capable of accumulating up to more than 40% of the total fatty acids content in DHA (Sahin et al., 2018). Other genera such as *Thraustochytrium* and *Labyrinthula* have also been identified as producers of DHA (Raghukumar, 2008). More generally, the PUFAs-producing microalgae species are *Phaeodactylum tricorutum*, *Monodus subterraneus*, *Porphyridium cruentum*, *Chaetoceros calcitrans*, *Nannochloropsis sp.*, *Cryptocodinium cohnii*, *Isochrysis galbana*, and *Pavlova salina* (Bhalamur et al., 2018; Mourelle et al., 2017).

3.3.2. Pigments

In addition to PUFAs, microalgae include photosynthetic pigments in their composition. The latter are classified into three groups: chlorophylls, carotenoids and phycobiliproteins responsible for green, yellow/orange and red/blue colours, respectively (Hamed, 2016; Siqueira et al., 2018). For several years, these compounds have been shown to have beneficial health properties, such as antioxidant properties, vitamin precursors, immune activators and anti-inflammatory agents (Borowitzka, 1995; Hamed, 2016). Therefore, they are mainly used for food, pharmaceutical or cosmetic applications as natural colours, food supplements or as a source of bioactive molecules (García et al., 2017).

- **Chlorophylls:** Chlorophylls are green pigments, essential for photosynthesis, present in almost all photoautotrophic organisms. Due to their high green pigmentation and growing consumer demand for more natural products, chlorophylls are gaining in importance as a dye agent in the food industries as well as in pharmaceuticals and in cosmetics (Odjadjare et al., 2017; Yaakob et al., 2014). Naturally present in photosynthetic organisms as chlorophyll *a* and chlorophyll *b*, these molecules can also be found in the form of sodium and cupric derivatives, for example. The latter are mainly used as food additives or in beverages (Yaakob et al., 2014).

Although chlorophylls are mainly extracted from inexpensive sources such as grass or alfalfa, microalgae contain a significant amount of them. The latter are therefore considered as an alternative source to chlorophyll extraction (Odjadjare et al., 2017; Yaakob et al., 2014), which would be potential for covalorization in the scope of biofuel production. Indeed, the chlorophyll content of a cell varies according to environmental conditions as well as the strain from about 0.5 to 4% of dry weight (Harun et al., 2010; Spolaore et al., 2006). A review highlighted the chlorophyll-inducing conditions in microalgae among which are low light, red light spectrum, a sufficiently high temperature, and a replete nitrogen and phosphorus content (da Silva Ferreira and Sant'Anna, 2016). To illustrate this, many studies have been carried out. As an example, a study showed that under high light intensity (400 $\mu\text{mol photon/m}^2/\text{s}$) the chlorophyll *a* content of two microalgae strains underwent a greatly decline compared with low light intensity (40 $\mu\text{mol photon/m}^2/\text{s}$) (He et al., 2015). *Chlorella sp.* decreased its

chlorophyll *a* content from 14 mg/L to 2 mg/L and *Monoraphidium* sp. from 11 mg/L to 3 mg/L. Another study assessed the effect of nitrogen depletion and low light intensity on the chlorophyll content of *Scenedesmus dimorphus* (Ferreira et al., 2016). The authors found a higher chlorophyll content when the cells were cultivated under low light intensity at 16.9 $\mu\text{mol photon/m}^2/\text{s}$ (128% higher than the control group grown at 123 $\mu\text{mol photon/m}^2/\text{s}$). Conversely, nitrogen starvation induced an abrupt decrease in chlorophyll content of about 77.53% compared to the initial time. In addition, the proportion of chlorophyll *a* and chlorophyll *b* within the cells was investigated. Microalgae exposed to low light intensities were found to have the same pigment distribution as the control group (81% of chlorophyll *a* and 19% of chlorophyll *b* average percentage relative to the total chlorophyll content). However, nitrogen deprivation induced the production of chlorophyll *b* at the expense of chlorophyll *a* (a decrease in 8.77% in chlorophyll *a* content and an increase by 42.21% in chlorophyll *b* content). In addition to nutrient status and light intensity, the impact of the visible light wavelengths (color spectrum) on the photosynthetic pigment production was also investigated. In this respect, a study emphasizes a difference in pigmentation according to light exposure for the two strains studied (Mohsenpour et al., 2012). The green microalgae *Chlorella vulgaris* was found to have an increased pigmentation and the highest chlorophyll content (1.29% and 0.38% dry weight for chlorophyll *a* and *b*, respectively) under red light of 650–700 nm compared to the daylight control (0.86% and 0.28% dry weight for chlorophyll *a* and *b*, respectively). Green light yielded the best chlorophyll *a* production in the cyanobacteria *Gloeotheca membranacea* with a chlorophyll *a* content of 1.23% dry weight compared with the control at 1.09% dry weight. Light photoperiod is another parameter that can be used to manipulate the cell chlorophyll content (Gonçalves et al., 2019). As an example, in *Chlorella vulgaris*, total chlorophyll content exposed to 60-s periods (215 $\mu\text{mol quanta/m}^2/\text{s}$ average light intensity) underwent a 50% increase compared to the continuous light reference (Levasseur et al., 2018).

Once the chlorophylls were extracted, the remaining cell fragments can be used to produce biofuels, for example. Among the most exploited strains, the most well known microalgae is from the genus *Chlorella*, whose chlorophyll content represents about 7% of its biomass, five times more than that of *Arthrospira* (Khanra et al., 2018).

- **Carotenoids:** Carotenoids are another class of pigments that are abundantly found in microalgae. These richly coloured molecules ranging from yellow to red are mainly known for their antioxidant property as well as dyeing power (Berthon et al., 2017; Odjadjare et al., 2017; Yaakob et al., 2014). Thanks to their properties, carotenoids are frequently encountered in food and feed industries as well as cosmetics and pharmaceutical area (Siqueira et al., 2018). With more than 600 representatives, carotenoids form the most diverse and widespread class of pigments (Gong and Bassi, 2016; Odjadjare et al., 2017). Most of them share a common chemical structure composed of a 18-carbon conjugated double bond chain with two hexacarbon rings at each end (Odjadjare et al., 2017). Within this large class, carotenoids are divided into two groups: carotenes and xanthophylls. The former are oxygen-free hydrocarbons such as α -carotene and β -carotene, while the latter are oxygenated derivatives of carotenes (lutein, violaxanthin, zeaxanthin, fucoxanthin, astaxanthin, among others) (Lee, 2018). The main sources of carotenoids are microalgae belonging to the Chlorophyceae class. These microalgae are capable of producing a wide range of pigments: carotenes (β -carotene, lycopene) as well as xanthophylls (astaxanthin, violaxanthin, anteraxanthin, zeaxanthin, neoxanthin and lutein, among others). Other pigments, such as fucoxanthin, diatoxanthin and diadinoxanthin are produced by other microalgae phyla (Berthon et al., 2017). On average, carotenoids represent 0.1 to 0.2% of the dry matter (Spolaore et al., 2006). However, under unfavourable conditions, these pigments can be

expressed up to 12% in the phylum Chlorophyta (Lee, 2018). Currently, the two pigments having the highest demand in global carotenoid market are β -carotene and astaxanthin from the genera *Dunaliella* and *Haematococcus*, respectively (Berthon et al., 2017; Milledge, 2011; Rammuni et al., 2019).

β -carotene is the first ever high-value product to be commercially produced from microalgae (Rammuni et al., 2019). It is commonly produced by *Scenedesmus almeriensis*, *Dunaliella bardawil* and *Dunaliella tertiolecta* (Berthon et al., 2017; Bhalamur et al., 2018; Borowitzka, 1995; Hamed, 2016; Khanra et al., 2018; Mourelle et al., 2017; Yaakob et al., 2014). However, the richest source for commercial production of natural β -carotene is the microalgae *Dunaliella salina* which is capable of expressing up to 98.5% β -carotene in relation to its total carotenoids and about 13% of its dry biomass (Molino et al., 2018a Rammuni et al., 2019). Although the latter is in competition with its synthetic equivalent, natural β -carotene would have the advantage to be the unique source of 9-cis β -carotene. This isomer plays a major role in quenching oxygen free radicals and preventing oxidative damage to the cell (Rammuni et al., 2019).

Astaxanthin, a red xanthophyll pigment, is the second most industrially exploited carotenoid. It is naturally produced by the microalgae *Chlorella zofingiensis*, *Chlorococcum* sp. and *Scenedesmus* sp. as well as the yeast *Xanthophyllomyces dendrorhous* (Bhalamur et al., 2018; Odjadjare et al., 2017; Yaakob et al., 2014). However, the microalga *Haematococcus pluvalis* (Hamed, 2016; Siqueira et al., 2018) is able to accumulate up to 81% of astaxanthin of its total carotenoids and about 7% of dry weight (Molino, Iovine, et al., 2018). Therefore, this microorganism is seen as the most favourable specie for industrial scale production of natural astaxanthin (Rammuni et al., 2019; Raposo et al., 2015). Due to its high red dyeing power, this carotenoid pigment is mainly used in aquaculture feed as a dye agent for fish and shellfish flesh (Hamed, 2016; Siqueira et al., 2018; Yaakob et al., 2014). However, the use of astaxanthin is not limited to its pigmentation. It is particularly known to have antioxidant properties ten times greater than other carotenoids, making it the most potent natural antioxidant (Berthon et al., 2017; Borowitzka, 1995; Hamed, 2016; Mourelle et al., 2017). In this respect, astaxanthin is linked to biological activities with positive effects on human health. However, despite its attractive biological properties, the cost of production of natural astaxanthin is higher than that of synthetic astaxanthin (2500 to 7000\$/kg and 1000\$/kg, respectively) (Molino, Rimauro, et al., 2018), which is a barrier to the industrial exploitation of this microalgae (Bhalamur et al., 2018).

Lutein is another yellow carotenoid found in microalgae. It is mainly involved in the formulation of drugs and cosmetics. Particularly, they are mainly known to have protective activity against photoinduced damage to the lens and retina of eyes (Roberts and Dennison, 2015). Currently, most of the lutein production comes from marigold flowers (lutein content in marigold granules: 20 g/kg) (Bhalamur et al., 2018; Lin et al., 2015; Yaakob et al., 2014). However, lutein production from microalgae is gaining more and more ground due to higher productivity (Bhalamur et al., 2018). Indeed, although they have a lower lutein content than flowers (on average 5 g/kg), the annual production of microalgae is estimated to be 70–150 tons/ha, 11.5–25 times higher than that of marigold flowers (6 tons) (Lin et al., 2015). Lutein-producing strains include *Muriellopsis* sp., *Chlorella protothecoides*, *Chlorella zofingiensis*, *Chlorococcum citrifforme*, *Neosporangium gelatinosum* and *Scenedesmus almeriensis* as the largest producers with the ability to produce up to 0.5% lutein on a dry basis (Bhalamur et al., 2018; Molino, Rimauro, et al., 2018; Yaakob et al., 2014).

Other carotenoid pigments with industrial applications include lycopene, violaxanthin and zeaxanthin. Lycopene is a natural red antioxidant found in the cosmetics industry as a sunscreen and in anti-aging care formulations (Bhalamur et al., 2018; Borowitzka, 1995). It is also used for pharmacological purposes because of its anti-carcinogenic and

antiatherogenic properties (Bhalamur et al., 2018). Violaxanthin, an orange carotenoid pigment, is particularly known for its anti-inflammatory and anti-cancer properties. It is naturally produced by the strains *Chlorella ellipsoidea* and *Dunaliella tertiolecta* (Berthon et al., 2017; Mourelle et al., 2017). Zeaxanthin is a yellow carotenoid. It is mainly used in the pharmaceutical, cosmetic and food industries. For its production, the strains *Scenedesmus almeriensis* and *Nannochloropsis oculata* are used (Bhalamur et al., 2018; Hamed, 2016). Finally, other carotenoids such as canthaxanthin, β -cryptoxanthin or fucoxanthin are used for pharmaceutical or cosmetic purposes because of their tanning, anti-inflammatory and anti-cancer properties, respectively (Borowitzka, 1995).

- **Phycobiliproteins:** Phycobiliproteins are the last class of pigments found in photosynthetic organisms. They are hydrophilic protein-pigments complexes present only in cyanobacteria, and microalgae of the Rhodophyta phylum as well as in some Cryptophytes and Glaucophytes (Arad and Yaron, 1992; Gouveia et al., 2010; Lee, 2018). Like chlorophylls *b/c/d/f* and carotenoids, phycobiliproteins are accessory photosynthetic pigments used to extend the range of light absorption needed for photosynthesis (from 450 to 650 nm) (Lee, 2018; Manirafasha et al., 2016; Masojidek et al., 2013). They are classified into four major subgroups according to their absorption spectra (Bhalamur et al., 2018; Odadjare et al., 2017): the red phycoerythrin (PE, λ_{max} = 540–570 nm), the magenta phycoerythrocyanin (PEC, λ_{max} = 560–600 nm), the blue phycocyanin (PC, λ_{max} = 610–620 nm), and the light blue allophycocyanin (APC, λ_{max} = 650–6500 nm). Their composition varies with the species and environmental conditions. Under certain conditions, they can represent up to 13% of the dry biomass of some microalgae (Arad and Yaron, 1992). As a matter of fact, researchers have investigated the effects of different culture conditions (light source and growth media) on the expression of phycobiliproteins of *Pseudanabaena mucicola* freshwater cyanobacteria (Khatoun et al., 2018). The highest total phycobiliprotein content (237 mg/g) was reached when the cyanobacteria was cultured on wastewater under white light. When zooming in on the different pigments individually, it appeared that phycocyanin was produced in larger quantities in blue light on BBM medium (0.419 mg/L), while allophycocyanin had better performance under white light on wastewater (0.523 mg/L). The phycoerythrin content was mainly influenced by changes in pH rather than light. As a result, its content in the study was found to be very low (> 0.05 mg/L). However, another study aiming at maximizing its content in *Porphyridium marinum* found the greatest amount to be 40 mg/g dry weight under 70 $\mu\text{mol photons/m}^2/\text{s}$, NaNO_3 = 3.4 g/L and metal solution = 1.5 mL/L (Gargouch et al., 2018). Hence, strategies need to be investigated to optimize phycocyanin production. In this regard, a study aimed at identifying the optimal growth conditions to obtain the highest phycocyanin content from *Spirulina platensis* (Ho et al., 2018). To do so, they set the following parameters: light intensity = 400 $\mu\text{mol photons/m}^2/\text{s}$, CO_2 aeration = 2.5% and flow rate = 0.2 vvm. Then, they varied different parameters such as the source of the illumination, the photoperiod, the nature of the culture medium. They finally found the highest phycocyanin content and productivity (14.9% and 101.1 mg/L/d, respectively) under white LED light source, 30:30 min light/dark cycles, recycled medium (50% replacement), and nitrate addition (45 mM).

Due to their powerful and highly sensitive fluorescent properties, phycobiliproteins are used as markers for certain immunological methods, in flow cytometry, microscopy and DNA tests (Hamed, 2016; Milledge, 2011; Odadjare et al., 2017; Siqueira et al., 2018; Yaakob et al., 2014). On an industrial scale, these pigments are produced from the species *Porphyridium sp.*, *Arthrospira sp.* and *Aphanizomenon flos-aquae* (Bhalamur et al., 2018; Borowitzka, 1995; Hamed, 2016;

Mourelle et al., 2017; Odadjare et al., 2017; Yaakob et al., 2014).

3.3.3. Carbohydrates

Carbohydrates are synthesized intracellularly and represent the major part of the compounds derived from photosynthesis. Their content can reach up to 50% of dry weight of certain microalgae (Yen et al., 2013). Among the genera and species most commonly used for the production of polysaccharides extracted from microalgae are *Tetraselmis sp.*, *Isochrysis sp.*, *Porphyridium cruentum*, *Porphyridium purpureum*, *Chlorella sp.* and *Rhodella reticulata* (Mourelle et al., 2017).

Once synthesized, these carbohydrates can serve as a variety of biological functions. Depending on their physiological role, polysaccharides are usually grouped into three classes: energy reserve polysaccharides, structural polysaccharides that participate in cell wall formation and polysaccharides involved in cell communication (Bernaerts et al., 2019; Pignolet et al., 2013). From a biotechnological point of view, only the first two categories of polysaccharides have industrial potential and are therefore developed in the following paragraphs. Furthermore, energy reserve polysaccharides have been studied in great depth mainly in the context of biofuel production. While structural polysaccharides show great promises for pharmaceutical, cosmetic and food applications thanks to the diversity of molecules, the studies dealing with them are very recent and remain few.

- **Energy reserve polysaccharides:** Carbohydrates, as storage compounds, provide energy for the metabolic processes of organisms and allow, if necessary, temporary survival in the dark (Markou et al., 2012). The synthesis of these reserve polysaccharides is a species-dependent process. Generally, polymers used as a storage product differ from those used as components of cell walls. In most microalgae species, the energy reserves are in the form of starch. The latter is a complex carbohydrate composed of amylose and amylopectin, two glucose polymers. Its degree of polymerization as well as its location within the cell differ according to the species of microalgae. In red microalgae, the Rhodophytes, starch is called floridian, i.e. it is stored as a vesicle in the cytoplasm outside the chloroplast. In green microalgae, the Chlorophyceae, starch is stored intraplasmically. In addition to starch and floridean starch, other types of storage polysaccharides including chrysolaminarin, paramylon and glycogen can be found (Bernaerts et al., 2018; Bernaerts et al., 2019). Although most microalgae store their reserves as starch, cyanobacteria tend to accumulate glycogen, sucrose or glucosylglycerol (González-Fernández and Ballesteros, 2012; Markou et al., 2012).

The constitution of energy reserves is not equivalent in all microalgae. For example, the strain *Porphyridium cruentum* is able to accumulate carbohydrates up to 57% of its dry matter content while *Chlamydomonas sp.* does not exceed 17% (González-Fernández and Ballesteros, 2012; Markou et al., 2012). As pointed out earlier, carbohydrates content can be manipulated through growth conditions. As a consequence, highly fluctuating carbohydrate levels have been reported in literature. For instance, *Chlorella vulgaris* may be able to accumulate carbohydrates between 9 and 41% of its dry matter content depending on growth conditions (Dragone et al., 2011; González-Fernández and Ballesteros, 2012). The same is true for *Scenedesmus obliquus*, which could accumulate between 10 and 47% of carbohydrates in its dry matter content (González-Fernández and Ballesteros, 2012). However, in literature, an antagonistic mechanism to the accumulation of carbohydrates has also been reported. Several studies have documented a conversion of carbohydrates to lipids when cells were exposed to different stress conditions. In this respect, a study showed that a salinity of 1 g NaCl/L induced a 58.7% conversion of carbohydrates to lipids in a microalgal consortium (Venkata Mohan and Devi, 2014). Temperature is another abiotic factor that can induce metabolic changes. Studies have shown that a temperature increase to 30 °C and 40 °C caused the

conversion of 57.8% and 42% of carbohydrates to lipids, respectively (Han et al., 2016; Venkata Subhash et al., 2014).

The main application of reserve polysaccharides is biofuels (Yen et al., 2013). Indeed, their ability to be easily digested without requiring pre-treatment makes them a good choice for biomass conversion technologies, in particular for anaerobic digestion. However, in order to maximize biofuel production from microalgae, it is necessary to combine the high innate carbohydrate content of a species with its ability to produce biomass in a significant way (Markou et al., 2012).

- **Structural polysaccharides:** Structural polysaccharides are gaining increasing importance in literature. These cell wall related polysaccharides were firstly exploited for their rheological properties as thickening or gelling agents. Yet they have recently received much attention because of the detection of different biological activities well reviewed (Raposo et al., 2013) in addition to being relatively easy to extract (Bernaerts et al., 2019; Pignolet et al., 2013). Consequently, these macromolecules can find applications in the food (de la Noue and de Pauw, 1988; García et al., 2017; Siqueira et al., 2018; Venugopal, 2016), cosmetics (Martins et al., 2014; Mourelle et al., 2017; Yen et al., 2013) and pharmaceuticals (Ahmed et al., 2014; Berthon et al., 2017; García et al., 2017; Mourelle et al., 2017; Siqueira et al., 2018; Venugopal, 2016; Yen et al., 2013) industries. Some polysaccharides are also thought to have biosurfactant activities used in bioremediation of water and soil (Siqueira et al., 2018).

Except for a few species, the microalgal cell is surrounded by a cell wall whose composition differs within a phylum, class or even species (Bernaerts et al., 2019). It is generally rigid, homogeneous and multi-layered. In eukaryotic microalgae, the cell wall consists of microfibrils embedded in a mucilage composed in part of polysaccharides, called fibrillar polysaccharides, such as cellulose and hemicellulose. A mixture of others polysaccharides such as xylose, mannose or sulfated polysaccharides are also found in different proportions. All these polysaccharides are related to the rigidity of the cell wall as well as the resistance to mechanical disruption techniques (Bernaerts et al., 2019). Unlike eukaryotic microalgae, cyanobacteria have a peptidoglycan-based cell wall made up of *N*-acetylglucosamine and *N*-acetylmuramic acid residues connected by β -1,4 linkages that are easily breakable (Bernaerts et al., 2019; D'Hondt et al., 2017).

Furthermore, several eukaryotic and prokaryotic microalgae can produce and excrete extracellular polymeric substances either non-covalently bonded to the surface of cells or in their surrounding environment. Most of the time, these polymers are referred to as exopolysaccharides although the terminology remains unclear (Chen et al., 2013; Siqueira et al., 2018; Yen et al., 2013). Contrarily to both intracellular and cell wall polysaccharides, these exopolysaccharides have a very complex structure. As a result, there is still limited information available on the structural characterization of carbohydrates from microalgae (Delattre et al., 2016). This phenomenon is all the more amplified by the fact that the interest in exopolysaccharides is rather recent (Bernaerts et al., 2019; Delattre et al., 2016; Pignolet et al., 2013; Templeton et al., 2012). However, several potential functions have been identified. Exopolysaccharides are known to promote the formation of cell adhesion or aggregates playing a role in the formation of biofilms (Bernaerts et al., 2019; Pignolet et al., 2013). In addition, their expression would be an adaptive response of microalgae to both mechanical and environmental stresses providing a protection to the cell. It is therefore not surprising that microalgae have been recognized as a good source for exopolysaccharides production ranging from about 0.5 g/L to up to 20 g/L (Delattre et al., 2016). However, to date, only few studies have been done on the subject. It also appears that, most of the time, the name used in literature does not allow to distinguish between exopolysaccharides and cell wall related polysaccharides making comparison difficult (Delattre et al., 2016).

Carbohydrates from microalgae are generally made up of a mixture

of neutral sugars, amino sugars and uronic acids in their structure. Their nature is species-dependent and therefore vary from one species to another (Geresh and Arad, 1991; González-Fernández and Ballesteros, 2012; Templeton et al., 2012; Venugopal, 2016; Xiao and Zheng, 2016). However, the 12 most common monosaccharides constituting the carbohydrates in microalgae are glucose, rhamnose, xylose, mannose, galactose, fucose, arabinose, ribose, glucosamine, galactosamine, glucuronic acid and galacturonic acid (Bernaerts et al., 2019; Siqueira et al., 2018; Templeton et al., 2012). Their detection is not always easy as their synthesis depends on the culture conditions, as well as the growth phases. One of the most commonly used strategies for the accumulation of exopolysaccharides is nutrient limitation. A study investigated the influence of different nitrogen sources on exopolysaccharide synthesis by *Botryococcus braunii* UC 58 (Lupi et al., 1994). The results highlight the strain ability to produce exopolysaccharides regardless of the nitrogen source but at different concentrations (2.4 g/L with nitrate, 1.8 g/L with urea and 2.1 g/L with ammonium after 14 days of culture). However, the nitrogen limitation had no effect on the composition of *Tetraselmis suecica* cell wall (Kermanshahi-pour et al., 2014). Regardless of the initial nitrate concentration (62 or 90 ppm), the cell walls were found to have a similar monosaccharide composition consisting of 54% 3-deoxy-D-manno-oct-2-ulosonic acid (Kdo), 17% 3-deoxy-lyxo-2-heptulosaric acid (Dha), 21% galacturonic acid and 6% galactose. However, nitrate depletion to a concentration of 3 ppm induced an accumulation of intracellular starch up to 45% of dry biomass.

Apart from culture media, physico-chemical culture parameters such as light, temperature, aeration can affect the exopolysaccharides composition and production. In this context, a study demonstrated that the production of exopolysaccharides from *Arthrospira platensis* was a light-dependent process and was also influenced by the temperature (Trabelsi et al., 2009). The authors found an optimum for exopolysaccharides production at light intensity higher than 180 $\mu\text{mol photons/m}^2/\text{s}$ and temperature at 35 °C. In addition to light intensity, wavelengths and therefore light spectrum also influence the production and monosaccharide composition of exopolysaccharides without changing their structure. A study conducted on *Nostoc flagelliforme* revealed that blue light was more suitable than white light for exopolysaccharides production (Han et al., 2014). The effect of CO₂ concentration was also investigated on exopolysaccharides production (Cheng et al., 2015). The same aeration (2% of CO₂) was applied to four different strains of *Chlorella*. Within the same species, different polysaccharides profiles were found. *Chlorella vulgaris* and *Chlorella variabilis* showed a different content of total carbohydrates in their cell wall (48% and 33% of total carbohydrates in cell wall, respectively) but their neutral cell wall monosaccharides profiles were quite similar (about 14% of arabinose, 36% of rhamnose, 43% of xylose + galactose + mannose and 7% of glucose). Conversely, *Chlorella sorokiniana* and *Chlorella minutissima* had the same total cell wall carbohydrates content (about 30%) but presented different cell wall composition: 0% arabinose, 38% rhamnose, 58% xylose + galactose + mannose and 4% glucose; and 0% arabinose, 3% rhamnose, 74% xylose + galactose + mannose and 23% glucose, respectively.

To date, there is poor knowledge of the structure of exopolysaccharides. In order to maximize their biotechnological exploitation, it is necessary to have access to their structural information to establish relationships between structure and biological activity. For this, further studies are necessary and expected in the coming years.

3.3.4. Proteins, peptides and amino acids

Depending on the species and environmental factors, the protein content varies from 6 to 70% of their dry weight, although the majority of them have protein levels around 50% (Becker, 2007). Microalgae also have a rich and varied composition of amino acids, among which the most abundantly found are aspartate (Asp) and glutamate (Glu) (Conde et al., 2013). These molecules have different biological

functions that can be used in the nutritional, cosmetics and pharmaceuticals fields.

Proteins from microalgae are mainly used as nutraceuticals or included in the formulation of functional foods (Khanra et al., 2018; Siqueira et al., 2018). Due to the high protein content of microalgae, mainly in *Arthrospira*, *Chlorella* and *Dunaliella salina*, they are considered to be an unconventional source of protein for human consumption, particularly for developing countries (Becker, 2007; Christaki et al., 2011; Kim and Kang, 2011). It would be estimated that by 2054, about 50% of the replacement protein market would be covered by proteins derived from algae or insects (Khanra et al., 2018). Furthermore, microalgae contain well-balanced essential amino acids profiles, however, they may have lower digestibility than standard protein sources (Becker, 2007; Bernaerts et al., 2019). In this respect, *in vitro* microalgae digestibility tests have been performed on different microalgae species. The results showed a large variability in protein digestibility values, from 50 to 82%, depending on the species analysed (Niccolai et al., 2019; Wild et al., 2018). In the high range, *N. sphaeroides*, *A. platensis* and *C. vulgaris* were found with crude protein digestibility values of 82%, 81% and 76%, respectively. These microalgae have a higher digestibility than some standard protein sources such as beans, oats and wheat (78%, 72% and 77%, respectively) (Niccolai et al., 2019). Conversely, other microalgae such as *N. oceanica* and *C. sorokiniana* exhibited relatively low protein digestibility values (50 and 55%, respectively). The reduced digestibility of some microalgae is explained by the composition of the cell wall. The thick, rigid and cellulosic cell wall in some microalgae is considered to be a barrier to digestibility (Kose et al., 2017). Conversely, cyanobacteria have a thinner cell wall that is more easily hydrolysable. Nevertheless, the digestibility of microalgae can be improved by exposing the cells to different processes (Kose et al., 2017). In this sense, a study has shown digestibility values of sixteen microalgae increased when the cells were subjected to a cell disruption process (Wild et al., 2018). In untreated cells, the highest average digestibility was found in *Chlorella sp.* (79%) and the lowest in *Nannochloropsis sp.* (54%). The process of cell disruption increased the average digestibility of all types of microalgae (78–84% on average), but with different gains. The largest increase was obtained in *Nannochloropsis sp.* (49% increase), while for the other genera, with already good digestibility, the increase was less significant: *Arthrospira sp.* (5%), *Chlorella sp.* (6%) and *Phaeodactylum sp.* (8%). In addition to nutraceuticals, proteins of microalgal origin would present promising technological functionalities for the agri-food industry. For example, *Chlorella vulgaris* and *Tetraselmis sp.* would have emulsifying and foaming properties (Bernaerts et al., 2019). Thickening or gelling properties have also been reported for *Arthrospira platensis* and *Tetraselmis suecica* proteins (Bernaerts et al., 2019).

Microalgal proteins, mainly from *Arthrospira* and *Chlorella* genera, have also penetrated the cosmetics field, and more particularly the skin market (Apone et al., 2019; Stolz, 2005). In general, protein extracts are found in the formulation of beauty products because of their attractive biological activities. These activities have already been well reviewed (Apone et al., 2019). At present, some beauty products containing protein extracts from microalgae can be found in the market. For example, Dermochlorella DG[®] is a product developed from *Chlorella vulgaris* extracts containing oligopeptides. This microalgae-based ingredient, by stimulating collagen production, acts as a restructuring agent that gives firmness to the skin, reduces the morphology of stretch marks and reduces vascular imperfections (Martins et al., 2014; Stolz, 2005; Yaakob et al., 2014). *Arthrospira* proteins are also used in cosmetics to repair the signs of early skin aging (Stolz, 2005). Micosporin-like amino acids have also attracted a lot of interest as potential cosmetic agents (Berthon et al., 2017; Mourelle et al., 2017). The latter specifically have photoprotective and antioxidant actions to fight against photoinduced skin aging (Mourelle et al., 2017; Siqueira et al., 2018).

From a pharmacological point of view, peptides from microalgae

have gained more attention as alternative bioactive compounds due to their safety status. They are mainly known to have anti-inflammatory (Vo et al., 2013), antihypertensive, anticancer (Sadeghi et al., 2018), antibacterial and antioxidant (Sheih et al., 2009) properties that can be applied in human health promotion (Galasso et al., 2019). These peptides are generally obtained following enzymatic hydrolysis of various proteins (Ejike et al., 2017; Kim and Kang, 2011). Proteins usually contain 3–20 amino acid residues, and their activity depends on their amino acid composition and sequence (Kim and Kang, 2011). One of the main producers of peptides is *Chlorella sp.* (Ejike et al., 2017). As such, many studies have been conducted on this genus. One of them was performed *in vitro* on hydrolyzed proteins from *Chlorella ellipsoidea* (Ko, Kim, and Jeon, 2012). This study identified a specific pentapeptide (Leu-Asn-Gly-Asp-Val-Trp) showing to have peroxy radical, 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and hydroxyl radicals scavenging antioxidant activities at the half maximal inhibitory concentrations (IC₅₀) values of 0.02, 0.92 and 1.42 mM, respectively. Once purified, the peptide was cultured with monkey kidney cells. The latter has improved both cellular viability against hydrochloride-induced cytotoxicity on normal cells (56.3%, 72.3%, and 79.4% at the concentrations of 25, 50, and 100 µM, respectively) and reduced the proportion of necrotic apoptotic cells hydrochloride-induced by 32.95%. The same author conducted another study on *Chlorella ellipsoidea* hydrolysates in order to identify anti-hypertensive properties (Ko, Kang, et al., 2012). In this respect, another peptide (Val-Glu-Gly-Tyr) was reported to have both *in vitro* angiotensin I-converting enzyme (ACE) inhibitory activity (IC₅₀ value = 128.4 µM) and *in vivo* systolic blood pressure lowering effects when orally administered in rats. A similar study has been conducted on both *Chlorella vulgaris* and *Spirulina platensis* peptides showing different peptides with different inhibition activity (Suetsuna and Chen, 2001). In *Chlorella vulgaris*, 4 peptides of the 5 peptides studied inhibited the ACE activity with lower IC₅₀ than in the previous study: Ala-Phe-Leu (63.8 µM), Phe-Ala-Leu (26.3 µM), Ala-Glu-Leu (57.1 µM), and Val-Val-Pro-Ala (79.5 µM). Five peptides have been identified from *Spirulina platensis*: Ile-Ala-Glu (34.7 µM), Phe-Ala-Leu, Ala-Glu-Leu, Ile-Ala-Pro-Gly (11.4 µM), and Val-Ala-Phe (35.8 µM). *In vitro* cytotoxic effects of *Dunaliella salina* peptides have also been reported in literature (Darvish et al., 2018). The antimicrobial activity was performed against *Escherichia coli*, *Staphylococcus aureus* and *Helicobacter pylori*. The highest inhibitory effect was obtained from peptides with > 10 kDa molecular weights for *E. coli* and peptides with 3–10 kDa molecular weights after 16 h of culture (16.8% and 17.2% inhibitory effect, respectively). The bioactive peptides with highest bacterial inhibition effect were observed on *H. pylori* culture with a minimum inhibitory concentration of 0.175 mg compared to *E. coli* and *S. aureus* (0.58 and 0.81 mg, respectively). Antiproliferative properties of *Dunaliella salina* peptides was analysed on human colon cancer cell lines (SW480). Among the three peptide fractions and concentrations studied, the most effective concentration appeared to be the peptides < 3 kDa in 0.1 µg/mL concentration after 72 h with about 30% viability. Although a large number of studies have been conducted using *in vitro* and cell-based assays for identifying properties, there are an increasing number of *in vivo* studies, mainly in rats. To our knowledge, the latest one to date was on the oral toxicity of microalgal protein hydrolysates on Wistar rats. This study has revealed the safety of these hydrolysates and thus supports their use in functional foods or nutraceuticals (Barkia et al., 2019).

These proteins, once synthesized, require extraction and purification steps. These must be monitored and tested to ensure that no changes in protein integrity occur as a result of these treatments (Dixon and Wilken, 2018).

3.3.5. Vitamins

Microalgae are an essential primary food source in the rearing of all stages of marine bivalve molluscs, larvae of several marine fish species and shrimps, and zooplankton providing them with nutritional intakes.

In addition to their balanced macronutrient composition, microalgae provide them with vitamins essential to their development as they are not able to synthesize them. These bioactive compounds are important metabolites because of their precursor properties of some important enzyme cofactors. Besides, they play an essential antioxidant role in the scavenger of ROS. Thus, the majority of studies on vitamin production by microalgae have been conducted on strains used in aquaculture.

In the context of aquaculture, marine microalgae are able of synthesizing and accumulating a wide range of vitamins including pro-vitamin A, some vitamins of the B group (B1, B2, B3, B5, B6, B8, B9 and B12), vitamin C, and vitamin E, among others (Galasso et al., 2019). The vitamin content from microalgae is both species-dependent (Brown et al., 1999; Coutteau and Sorgeloos, 1997; De Roeck-Holtzhauer et al., 1991; Fabregas and Herrero, 1990) and correlated with the growth phase and growth conditions (Brown et al., 1999). For instance, a study demonstrated that riboflavin (vitamin B2) content of six microalgae species used in mariculture all increased during the onset of stationary growth phase (Brown and Farmer, 1994). Later, the same author studied the effect of photoperiod and harvest stages on *Nannochloropsis* sp. CS-246 vitamins content (Brown et al., 1999). Under different light conditions, the content of most of the vitamins changed. Both vitamin B2 and B6 were found in larger quantities under constant light (62 and 9.5 µg/g, respectively compared with 25 and 3.6 obtained under light/dark cycles of 12:12 h) while pro-vitamin A content was found to be lower (0.29 compared with 0.50 mg/g). Harvest stage also affected the vitamin content. Some vitamins such as vitamin C and vitamin E are expressed in greater quantities when the culture is harvested during the exponential phase. In contrast, the pro-vitamin A and vitamin B2 contents decreased as the culture grown and was harvested during the stationary phase. Another study also investigated the relationship between light availability and vitamin yield in *Dunaliella tertiolecta* (Barbosa et al., 2005). The vitamin C content of the cells increased from 1.72 to 3.48 mg/g with increasing average light intensity (from 84 to 430 µmol/m²/s). Unlike vitamin C, the authors found a negative correlation between the amount of vitamin E produced per mole of photons absorbed and the average light intensity inside the reactor. Two years earlier, a study had shown the same trend (Carballo-Cárdenas et al., 2003). However, the authors highlighted that vitamin E production was not only a function of light, but also of the strain and growth phase. Under the same conditions, *Tetraselmis suecica* expressed the highest vitamin E content during the exponential phase (1.08 mg/gDW). However, this content gradually declined over the rest of the growth to reach a final value of 0.4 mg/gDW. In contrast, in *Dunaliella tertiolecta*, the vitamin E content increased with increasing cell density to reach up to 0.5 mg/gDW at the end of the culture. Finally, the availability of nutrients in the culture medium is also likely to affect vitamin synthesis in microalgae. As an example, a study investigated the effects of the nitrogen source (sodium nitrate and ammonium chloride) and their concentration (882 and 441 µmol/L) on the accumulation of vitamin E in *Nannochloropsis oculata* (Durmaz, 2007). The authors identified the highest vitamin E content (2325.8 µg/g dry weight) at 441 µmol/L sodium nitrate in the late stationary phase, i.e. 34 days of culture suggesting that decreasing nitrogen concentrations led to an increase in vitamin E accumulation. All these results support that vitamin expression is a species-dependent process and is adjusted according to the environmental conditions to which they are exposed (light, nutrients) as well as the harvesting stage.

In addition to aquaculture, studies have also been carried out on the contribution of microalgae sourced vitamins to human nutrition. In this respect, commercially available microalgae powders, intended for consumption as source of vitamins B2, B3, B9 and B12, have been examined (Edelmann et al., 2019). The vitamin B2 content of *Spirulina* powders showed a low variability between the tested products, with a maximum content of 40.9 µg/g. Vitamin B3 is expressed more strongly in *Chlorella* (0.24 mg/g) than *Spirulina* (0.16 mg/g) powders, although the difference observed was not statistically significant. Nevertheless

these powders represent only a small portion of the recommended daily allowances of riboflavin (B2) and niacin (B3) for adults, which are 1.3 and 16 mg, respectively. In contrast, *Chlorella* and *N. gaditana* powders have been shown to be a good source of vitamin B9. Their maximum content (25.9 µg/g and 20.8 µg/g, respectively) is six times higher than that of *Spirulina* (4.7 µg/g). These quantities represent about a quarter of the recommended daily intake (400 µg) for a consumption of 5 g of microalgal powder. Moreover, another recent study has identified microalgae, in particular the cyanobacterium *Anabaena cylindrica*, as a non-toxic source of vitamin K1 (Tarento et al., 2018). This vitamin, which plays a role in the prevention of chronic diseases, is mainly produced chemically. Cyanobacterial production of vitamin K1 offers an interesting biotechnological alternative since it produces only the active isomer of the molecule, as opposed to 10 to 20% of inactive isomer by chemical means. Additionally, its very high concentration of around 200 µg/g dry weight (six times higher than the 37 µg/g found in parsley, a rich vitamin K1 food source) provides three times the daily needs of an adult for a 1 g intake of powder. Furthermore, others studies point out that the microalgal vitamin content is superior to certain vegetable commodities. Notably, some microalgae would have higher vitamin pro-vitamin A, vitamins E, B1 and B9 contents than conventional foods such as orange, carrot, wheat flour, corn flour, rye flour or soy flour (Fabregas and Herrero, 1990). Besides, fruits and vegetables do not represent a good source of vitamin B12 because the latter is neither synthesized nor required by the plants (Koyande et al., 2019). Consequently, a vegetarian or vegan diet can lead to a deficiency of this vitamin. To make up for this deficiency, microalgae are considered as an essential source of vitamin B12 for people following these diets (Koyande et al., 2019). For example, *Chlorella*-based powder can contain up to 2.4 µg/g of vitamin B12, thus covering five times the recommended daily intake of 2.4 µg for an intake of 5 g of powder (Edelmann et al., 2019). In addition, one gram of *Anabaena cylindrica* powder provides 64% of the adult vitamin B12 intake (Tarento et al., 2018). Still, the bioavailability of these vitamins is species-dependent (Galasso et al., 2019).

Thus, depending on the desired product, different strategies are to be implemented. Moreover, a special attention must be paid to post-harvesting treatments as drying processes. These latter could have a considerable effect on the vitamin content, especially on the heat unstable vitamins such as B1, B2, B3 and C.

3.3.6. Polyphenols

Polyphenols are a wide group of secondary metabolites comprising phenolic acids, flavonoids, isoflavonoids, stilbenes, lignans and phenolic polymers (Galasso et al., 2019). Their basic structure consists of one or more hydroxyl groups bound to an aromatic ring, making them polar compounds. These molecules display a wide range of biological activities including antioxidant activities as well as anti-inflammatory, anti-cancer, anti-allergic, anti-diabetes, anti-aging and antimicrobial properties (Galasso et al., 2019; Li et al., 2007). A recent review focusing on both preclinical and clinical studies of polyphenols from seaweeds suggests that these latter would improve cardiovascular-associated disorders, but the authors point out that further data are needed to make this clear (Gómez-Guzmán et al., 2018). Recently, a study was carried out on polyphenols extracted from two different microalgae species: *Nannochloropsis* sp. and *Spirulina* sp. (Scaglioni et al., 2019). These extracts were used to study antifungal and antimycotoxigenic properties on trichothecenes mycotoxins from *in vitro* cultures of *Fusarium graminearum*. This study revealed that after 168 h of culture, 40 µg/mL of phenolic extracts from *Nannochloropsis* sp. completely inhibited nivalenol and deoxynivalenol and dramatically hindered acetylates production by 98%. Polyphenol extracts from *Spirulina* sp. totally inhibited nivalenol and significantly decreased deoxynivalenol production by 62% and reduced acetylates contents by 78%.

Extensive information on polyphenols exist on macroalgae (Montero

et al., 2018), however, production of polyphenols from microalgae was also studied. Like all other active biological compounds, both the composition and content of polyphenol from microalgae are species-dependent. A screening of 32 different species was performed to determine the polyphenol contents (Goiris et al., 2012). From this study, it has been shown that polyphenols range between 54 mg Gallic Acid Equivalent (GAE)/100 g DW (*Haematococcus pluvialis*) to 375 mg GAE/100 g DW (*Phaeodactylum tricornutum*). *Tetraselmis* sp. and *Neochloris oleoabundans* are also great producers with 374 and 373 mg GAE/100 g DW, respectively. Other studies on microalgal polyphenols have found results within this range. For instance, a study found that *Arthrospira platensis* exhibits 334 mg GAE/100 g DW while *Chlorella vulgaris* expresses 217 mg GAE/100 g DW (Matos et al., 2019). Moreover, when compared to other sources of polyphenols, it has been observed that microalgae have total polyphenolic content similar to or higher than several vegetables and fruits (Manach et al., 2004). However, the greatest polyphenolic producers remain red berries.

Despite this step forward, further studies are needed to explore the production mechanism of polyphenols in more detail to determine the advantageous conditions to their expression.

3.3.7. Phytosterols

Sterols are components of the cellular membrane acting on its stability, permeability and fluidity by controlling movement of fatty acid chains within it (Ahmed et al., 2015; Galasso et al., 2019; Volkman, 2003). They are nitrogen-free complex polycyclic alcohols that originate from isoprenoid biosynthesis. Among the 200 reported phytosterols, β -sitosterol is the most abundant in higher plants, but in a number of microalgae it appeared that 24-ethylcholesterol was dominant (Ahmed et al., 2015; Galasso et al., 2019; Volkman, 2003). In addition, the sterols present in microalgae display both a great diversity of structures and occurrence variety. These compounds are species-dependent and their composition can also be influenced by environmental factors such as light intensity, temperature, salinity and growth stage (Le Goff et al., 2019; Volkman, 2003).

Phytosterols have already been the subject of numerous reviews because of their beneficial effects on human health (Ahmed et al., 2015; Kritchevsky and Chen, 2005). Particularly, they are well-known for their ability to reduce low density lipoprotein (LDL)-cholesterol and promote cardiovascular health with an intake of 2–3 g/days minimum. Besides, they present anti-inflammatory, anti-atherogenicity, anticancer and anti-oxidative activities (Woyengo et al., 2009). An *in vivo* study conducted on mice also highlighted some protection properties of β -sitosterol against nervous system disorders (Kim et al., 2008). In addition to their properties, the bioavailability of phytosterols has been studied by exploring their absorption on different animal species. It has been reported that rabbits do not absorb phytosterols while rats and humans absorb a small fraction (4% and 1.5% to 5%, respectively) (Kritchevsky and Chen, 2005).

In microalgae, a screening of 10 species commonly used in aquaculture and the nutraceuticals industry was conducted to determine the best candidates for phytosterols production (Ahmed et al., 2015). From this study, it emerged that *Pavlova lutheri*, *Tetraselmis* sp. M8 and *Nannochloropsis* sp. BR2 were the greatest producers of total phytosterols (from 0.4 to 2.6% dry weight). In addition, when stressed, *Pavlova lutheri* was able of expressing sterols up to 5% DW (day 14 at 35 salinity) (Ahmed et al., 2015).

Currently, the main industrial sources of phytosterols are tallow and vegetable oils. For instance, corn and rapeseed are capable to synthesize 850 mg/100 g and 820 mg/100 g of phytosterols, respectively (Ahmed et al., 2015). Microalgae are also good producers of phytosterols, especially *Pavlova lutheri* with 5186 mg/100 g of phytosterols. However, further efforts are needed to induce overexpression of phytosterols from microalgae through metabolic or genetic engineering, as well as on recovery and purification methods.

3.3.8. Hormones

Phytohormones are a class of molecules produced in very low concentrations serving as chemical messengers to coordinate cellular processes in higher plants (Lu and Xu, 2015). Most of the microalgal lineages are able to produce phytohormones. Among the classical phytohormones, there are auxin, abscisic acid, cytokinin, ethylene, gibberellins, polyamines, jasmonides, salicylates, signal peptides, and brassinosteroids (Bajguz, 2019; Lu and Xu, 2015; Mourelle et al., 2017). Although their functional roles are not well known to date, they have recently been studied (Lu and Xu, 2015; Mourelle et al., 2017). Depending on strains, phytohormones can act on development, growth, light response, stress tolerance, secondary metabolite synthesis and senescence of microalgae and thus regulating homeostasis to cope with variable environmental factors (Bajguz, 2019; Lu and Xu, 2015). As a result, understanding phytohormone pathways can be considered as a strategy for the selection of microalgae species for specific industrial purposes in particular for enhanced biofuel production (Lu and Xu, 2015). Indeed, the manipulation of phytohormone metabolism could increase biomass or secondary metabolites productivity by allowing better tolerance to adverse environmental conditions. In addition, microalgae-based hormones could play a role in counteracting the signs of skin aging in the field of cosmetics (Mourelle et al., 2017).

4. Current and projected market share

In 2017, the global market for microalgae-based products was estimated at 32.60 billion USD and is projected to reach approximately 53.43 billion USD by 2026 (Rahman, 2020). Currently, more than 75% of the production of microalgae-derived products is dedicated to food, feed or nutraceutical applications (Rahman, 2020), thus high value-added products can be considered as niches. Among those, the first established were dyeing agents, with 300 million USD in 2009, and nutraceuticals, with 30 million USD in 2009 (Rahman, 2020). Since then, other potential markets have emerged. As such, it is estimated that in 2020, dyeing agents will still be the leading microalgae product on the market (800 million USD), closely followed by pharmaceuticals/chemicals (500 million USD), nutraceuticals (300 million USD) and finally cosmetics (30 million USD) (Rahman, 2020). Currently, the market for microalgae-based products is dominated by the United States, Asia and Oceania. However, in the coming years, Europe is likely to become one of the leaders in the field of microalgae-based bioproducts (Rahman, 2020).

Two species, *Arthrospira* (trade name *Spirulina*) and *Chlorella*, are at the head of the world market with a production of 12,000 tons per year and 5000 tons per year, respectively (MU et al., 2019; Wang et al., 2020). These production figures are expected to increase further thanks to the growing consumer demand for a healthier diet. As an example, it is estimated that by 2050 more than 18% of protein sources will be derived from microalgae (Caporgno and Mathys, 2018). As a result, the increase of the market volume of *Spirulina* powder is estimated to reach 380 million USD by 2027, compared to 220.5 million USD in 2017 (MU et al., 2019).

If we take a closer look at the market shares of current microalgae products (Table 4), one can see that only certain high value-added molecules from microalgae have really made inroads into the world market. Among these molecules, one can find PUFAs, in particular omega-3 with a current market share of 2.5 billion USD in 2014, and carotenoids with 1.5 billion USD in 2017. Currently, the largest share of the PUFAs market is held by the United States. However, this market is expected to grow at an average annual growth rate of 13.5% worldwide (Rahman, 2020). The market for carotenoids is also growing steadily owing to the health benefits of these molecules. Of the more than 600 existing carotenoids, only two have achieved commercial: β -carotene and astaxanthin. β -carotene market was estimated to range between 224 and 285 million USD in 2019 (Rahman, 2020; MU et al., 2019). The global market for astaxanthin was estimated at 257 million USD in

2009 (Khattar et al., 2009), 555.4 in 2016 (Markets, n.d) and is projected to reach 1.1 billion USD in 2020 (Shah et al., 2016). Still, only 1% of this value corresponds to microalgae sourced astaxanthin. However, with the emergence of natural and organic products, the market value for natural astaxanthin can be expected to increase. In this perspective, it has been estimated that the market share of astaxanthin derived from microalgae would be 770 million USD by 2024. Provided proper market share increase, astaxanthin could be the flagship molecule driving microalgal biorefinery. Indeed, while natural astaxanthin prices range from 2500 to 7150 USD/kg (Kim et al., 2015; Koller et al., 2014), nutraceutical grade astaxanthin originating from *Haematococcus pluvialis* can reach 6000 USD/kg Dawidziuk et al. (2017), with production costs fluctuating between 300 and 3600 USD/kg (Li et al., 2011). Still, this enthusiasm has to be moderated as synthetic astaxanthin prices is around 1000 USD/kg (Li et al., 2011), positioning it as an economically sound alternative when biosourced counterpart is not required.

5. Discussion

From the proposed review, one can see that microalgae have a great potential for high added-value molecules production. Still, the levels of maturity of the aforementioned categories variate, while pigments and PUFAs have penetrated their markets, other molecules are still under active research. In particular, exopolysaccharides, polyphenols, phytoosterols and hormones are classes of molecules whose research wave is relatively recent. Further research efforts to better understand the structures and/or mechanisms of production are needed to hopefully make them emerging technologies in the coming years.

The combination of both technical and economical elements allows to take step back and analyse the current structure of the microalgal industry and draw projection from it. PUFAs production has been driven by the general public demand for DHA. This demand emanates from the recommendation of the WHO for both infant (minimum 0.3 gDHA + EPA/day) and adult health (0.250 gDHA + EPA/day) (J. F. E. C. on Fats and Fatty Acids in Human Nutrition, n.d). Still PUFAs market supply is ensured by fish oil, themselves originating from the microalgae fishes consume. This highlights the potency of general public organisations in market development. From a wider perspective, nutraceuticals will probably be the main driver of microalgal culture for high added-value molecules. This would shift the community position on the production of certain molecules. For example, while currently produced for their fish flesh dyeing properties, with this new driver in mind, β -carotene and astaxanthin would be produced for their health benefits. If microalgae-based chlorophyll were to achieve high market

volume, it would also be driven by nutraceuticals market and not dyeing agents ones. Phycobiliproteins are an exception to this observation.

In the nutraceuticals perspective, it is likely that microalgae would not be fragmented before being sold but marketed as a whole product, avoiding expensive fractioning costs. It would also allow retailers to promote health benefits of other biochemical compounds hosted by microalgae such as vitamins and proteins. Still proteins have a wider scope than sole nutrition. Microalgae-based proteins could be also driven by their technical properties appealing to the cosmetics and/or pharmaceutical sectors. Those two sectors will surely be the drivers of few microalgae applications with pin-pointed molecules. These applications could emerge from intrinsic value of the microalgal products, e.g. anti HIV, or from regulatory demands, e.g. plastic microbeads ban pushes the cosmetic sectors towards other texturing agents, microalgae polysaccharides could be among them. This last point highlights the tremendous power held by the regulator and how its coming positions will ease or not the rise of microalgal biorefinery into power.

With either nutraceuticals or cosmetics/pharmaceuticals focus, microalgae cultures will be product driven. Still subsequent capital will be needed for both culture protocol and product development, making the early choice of the relevant strain all the more relevant. In the light of microalgae diversity, this choice may not be an easy task. With this aim, Fig. 4 ties together classification, phyla specific bioactive compounds and their properties to biotechnological applications.

As one can see, Chlorophyta phylum is the one with the most diverse applications. It is followed by Cyanobacteria and Heterokontophyta phyla. Other phyla, usually cultivated for aquaculture (Cryptista, Haptophyta) seem to have potential for higher value application, such as PUFA. At the other end of the list, Euglenozoa phylum has not been identified as a high added-value application and seems to be restricted to wastewater treatment. In addition, to identify potential valorisation for a given strain, Fig. 3 highlights that some compounds can only be produced by a very restricted set of phyla. It is specifically the case for chlorophylls and carotenoids which are produced at relevant levels by Chlorophyta, or for technical proteins produced by Chlorophyta and Cyanobacteria. Still, no matter which molecule would be extracted, it could be valorised in different configurations thanks to several positive effects (except for vitamins whose have only one). Regarding applications, despite their diversity, the potential sectors of applications for microalgae extracted bioactive compounds mostly belong to cosmetics and pharmaceutical industries. Finally, food industry may consider microalgae for technical purposes restricted to dyeing and rheological agents.

Despite the benefits of microalgae, there are many bottlenecks

Table 4
Current and projected market share for microalgae-based products (N.A.: Not Available).

Products	Current demand	Projected demand	Main producing strains
Omega-3 PUFAs EPA	2.5 billion USD in 2014 (Matos, 2017) 300 million USD in 2010 (FAO, n.d)	5 billion USD by 2020 (Wang et al., 2020) 4 billion USD for EPA/DHA by 2022 (MU et al., 2019)	<i>Isochrysis galbana</i> , <i>Nannochloropsis oculata</i> , <i>Phaeodactylum tricoratum</i> <i>Cryptocodinium cohnii</i> , <i>Schizochytrium</i> <i>limacinum</i> , <i>Ulkenia</i> sp.
DHA	1.5 billion USD in 2010 (FAO, n.d)		
Carotenoids	1.5 billion in 2017 (Butler and Golan, 2020; Nwoba et al., 2020; Ruiz et al., 2016)	2.0 billion between 2019–2022 (Butler and Golan, 2020) (Nwoba et al., 2020)	
Astaxanthin	both synthetic and natural forms: 555.4 million USD in 2016 (Markets)	1.1 billion USD by 2020 (Shah et al., 2016) to 2.57 billion by 2025 (MU et al., 2019). Microalgae-based form: 770 million USD by 2024 (MU et al., 2019)	<i>Haematococcus pluvialis</i>
β -carotene	224–285 million in 2019 (Research, 2018; MU et al., 2019)	N.A.	<i>Dunaliella salina</i>
Phycobiliproteins	Greater than 60 million USD in 2010 (Wang et al., 2020)	N.A.	
Phycocerythrin	10–50 million USD in 2019 (Nwoba et al., 2020)	N.A.	<i>Porphyridium</i> sp. and <i>Rhodella</i> sp.
Phycocyanin	112.3 million USD in 2018 (MU et al., 2019)	232.9 million USD by 2025 (MU et al., 2019)	<i>Arthrospira</i> sp., <i>Porphyridium aeruginum</i>

throughout the process of their exploitation (Gifuni et al., 2019). During the culture stage, strain selection, which this article aims at helping, strain engineering and nutrient supply strategies need to be optimized for the hyperaccumulation of a single product. In addition, the growth of microalgae in culture is strongly limited by the degree of light penetration, resulting in dilute cultures with low biomass concentrations (about 3 g/L for autotrophic cultures compared to 30–100 g/L for heterotrophic fermentations). The diluted nature of the culture has major repercussions on processes downstream of the value chain, which represent 40% of the total costs of the entire process, and in particular on the harvesting stage (Fasaei et al., 2018). Finally, the extraction of metabolites of interest is also limiting due to the capacity of microalgae to effectively resist cell disruption processes due to the nature of the biochemical composition of their cell wall (Sialve et al., 2009; Ward et al., 2014). At present, these bottlenecks have not been resolved. The most optimistic projections envision a whole biomass cost of 0.62 €/kg dry weight (accounting for 0.20 €/kg dry weight cost reduction associated to wastewater treatment valorisation) (Acién Fernández et al., 2019). While possible, the growth of microalgae on wastewater with the aim of producing high added-value molecules seems unlikely. Indeed, even though it would, marginally, reduce the productions costs, the use of wastewater would induce undesirable/unpredictable variations in the growth substrate. Luckily, the diversity of applications widens the valorisation horizon as it is an edge that may alleviate the cost burden associated with biomass low cost (biofuel, feed) valorisation. Depending on the market value associated to the targeted bioactive compounds, two strategies can be envisioned. For

high price molecule such as astaxanthin, its sole hyperaccumulation may bring economic viability to the production process. In addition, after extraction of this flagship molecule, spend biomass can be turned to biofuel. An alternative strategy would be the production of two or more medium added value compounds without paying the additional production costs associated to hyperaccumulation promoting growth conditions (Gifuni et al., 2019).

Finally, one can note that, in recent years, genetic and metabolic engineering has emerged as a potential tool for promoting economical viability of microalgal biotechnology. Indeed, it allows to modify existing strains to improve their performances hence reducing associated production costs. Several examples can be given in this regard. First, in order to make the production of biofuels from microalgae profitable, strains can be modified to overexpress lipid production (Brennan and Owende, 2010; Hamed, 2016). Another relevant example is the production of recombinant proteins. A recombinant protein is a protein produced by a cell whose genetic material that has been modified by genetic recombination. Recombinant proteins obtained from microalgae include antibodies, vaccines and enzymes (Dixon and Wilken, 2018; Gong et al., 2011). Microalgae have been considered and studied as a platform for the production of these proteins. Being eukaryotic cells, their main advantage is to contain the cellular machinery allowing them to synthesize complex human proteins in a cost effective manner (Specht et al., 2010). Thanks to the complete sequencing of the genome of *Chlamydomonas reinhardtii*, the latter has great potential for the production of recombinant proteins (Specht et al., 2010; Gong et al., 2011; Dixon and Wilken, 2018). Other strains such as *Chlorella*

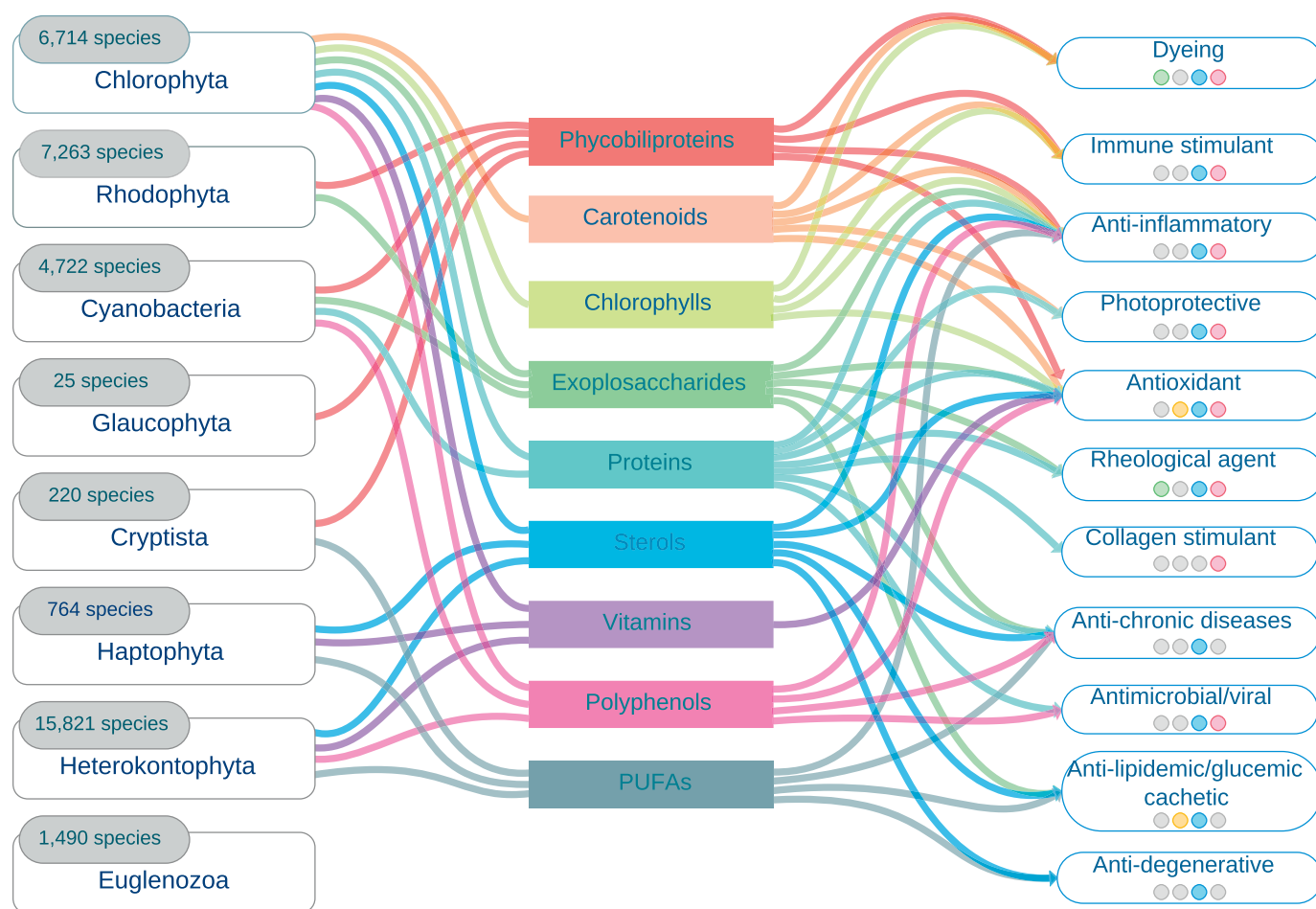


Fig. 4. Main bioactive compounds expressed in microalgae in relation to their classification and possible applications as high value added products. Numbers of species obtained from Guiry (2012); Guiry and Guiry (2020). Blue circle: pharmaceuticals; pink circle: cosmetics; yellow circle: nutraceuticals and green circle: food. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

ellipsoidea, *Dunaliella tertiolecta* or *Dunaliella salina* have also been studied (Dixon and Wilken, 2018). Some of the notable successes, although many, are the expressions of an anti-glycoprotein D antibody of herpes simplex virus, a monoclonal antibody IgG1 directed against anthrax protective antigen or the human protein TRAIL known to induce apoptosis in tumor cells infected with a virus (Dixon and Wilken, 2018; Gong et al., 2011; Specht et al., 2010). However, routine genetic manipulation has been limited to a few species until recently (Valverde et al., 2016). To date, few - about thirty - species have undergone transformations (Hamed, 2016; Pulz and Gross, 2004; Wijffels et al., 2013). As for other genetically modified organisms, modified microalgae would have to face two challenges regulations and consumer acceptance.

6. Conclusion and perspectives

This work reviewed applications of high added value molecules produced from microalgae. From this, it can be stated that, older forms of valorization - health food and quality feed, polyunsaturated fatty acids, pigments, carbohydrates - are currently penetrating their markets. For some they can even be found on supermarkets shelves. They are driven by two factors. For general public goods, the consumer appetite for healthy food and biosourced molecules in cosmetics. For industrial applications, desirable technical properties: e.g. texturer and dye for food industry, antioxidant for cosmetics. Most recent developments, such as peptides, vitamins, polyphenols, phytosterols and phytohormones, are struggling to meet their market and reach economical competitiveness. Still they are pushed forward by the very powerful driver that is pharmaceutical industry and the hope to gain new drugs and therapy. Regarding those, only time, research and development will tell us if they fulfill their expectations.

This work not only reviewed the aforementioned applications, it also tried to link microalgae classification and related potential applications. This is done through highlighting of which bioactive compounds can be found in which phyla. While some seem to be restricted to aquaculture, Cyanobacteria, Chlorophyta and Rhodophyta show great promises. This diversity of potential can be explained by two factors: the low number of strains in some phyla (25 and 220 species currently described for Glaucophyta and Cryptista, respectively), meaning low intrinsic potential, and the past direction of global research effort which may have biased our perception towards some phyla.

Finally, in addition to unravelling microalgae capabilities, academics and engineers still have to focus on alleviating large scale production bottlenecks, namely, high density cultures, harvest and molecules extraction. This could be done by strain genetic engineering, rising concerns about regulation and consumer acceptance, or conventional process optimization.

Declaration of author contributions

WL and VP initiated and planned the project. WL drafted the manuscript. PP and VP performed critical revision of the manuscript. All the authors read and approved the final manuscript and take responsibility for the integrity of the work as a whole, from inception to finished article.

Statement of Informed Consent, Human/Animal Rights

No conflicts, informed consent, human or animal rights applicable.

Declaration of Competing Interest

Authors have no conflict of interest to disclose.

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