

1 **Biological aspects and biotechnological potential of marine diatoms in relation to**
2 **different light regimens**

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4 **Costanza Baldisserotto¹, Alessandra Sabia¹, Lorenzo Ferroni and Simonetta Pancaldi***

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6 Department of Life Sciences and Biotechnology, University of Ferrara, C.so Ercole I d'Este, 32,
7 44121 Ferrara, Italy

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9 *Corresponding author: e-mail address, simonetta.pancaldi@unife.it; phone: +390532293786

10 ¹ Equal contribution

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12 **Keywords: biotechnological application, cultivation methods, light, marine diatoms**

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15 **Abstract**

16 As major primary producers in marine environments, diatoms are considered a ~~potentially~~
17 ~~new and~~ valuable feedstock of biologically active compounds for application in several
18 biotechnological fields. Due to their metabolic plasticity, especially for light perception and
19 use and in order to make microalgal production more environmentally sustainable, marine
20 diatoms are considered good candidates for the large-scale cultivation. Among physical
21 parameters, light plays a primary role. Even if sunlight is cost-effective, the employment of
22 artificial light becomes a winning strategy if a high-value microalgal biomass is produced.
23 Several researches on marine diatoms are designed to study the influence of different light
24 regimens to increase biomass production enriched in biotechnologically high-value

25 compounds (lipids, carotenoids, proteins, [polysaccharides](#)), or with emphasised photonic
26 properties of the frustule.

27

28 **Main characteristics of diatoms: a summary.**

29 Diatoms are eukaryotic unicellular photosynthetic micro-organisms, whose most peculiar
30 morphological feature is the typical cell wall (frustule), organised in biosilica valves (Round et
31 al. 1990). Diatoms were historically divided in two main groups, Centrales and Pennales, on
32 the basis of the frustule morphology: Centrales were marine diatoms characterised by circular
33 or polygonal valves, Pennales were freshwater diatoms with bilateral symmetry (Smol and
34 Stoermer 2010). Currently they are considered to belong to Heterokonta (or Stramenopiles)
35 in the SAR (Stramenopiles/Alveolaria/Rhizaria) monophyletic super-group (Burki et al.
36 2007), or Harosa (Cavalier-Smith 2018).

37 As Heterokonta, diatoms are characterised by plastids containing chlorophylls *a* and *c* and
38 carotenoids, such as β -carotene and xanthophylls, which confer the typical “gold-green”
39 colour to the cell (van den Hoek et al. 1995). The main carotenoid is fucoxanthin ([Lavaud](#)
40 [2007](#); Wilhelm et al. 2006; ~~Lavaud 2007~~). The peculiar molecular structure of this pigment
41 confers diatoms unique spectral properties with absorbing light in an extremely wide spectral
42 range, between 460 and 570 nm (blue to green/yellow lights) (Zigmantas et al. 2004). The
43 plastid is surrounded by four membranes and contains lamellae of elongated thylakoids
44 organized in bands of three, with no differentiation into stacked and unstacked regions
45 (Lavaud 2007). Thylakoids are surrounded by a typical “girdle lamella”, which is continuous
46 around the periphery of the plastid ([Lavaud 2007](#); Round et al. 1990; ~~Lavaud 2007~~). Plastids
47 contain at least one pyrenoid (Round et al. 1990). Due to the peculiar evolutionary origin
48 (Armbrust 2009), ultrastructure and organization of the photosynthetic apparatus of diatoms
49 greatly differ with respect to that of higher plants and other groups of algae ([Lavaud 2007](#);

50 Wilhelm et al. 2006, 2014; Lavaud 2007). Diatoms possess a large number of members of the
51 LHC (Light Harvesting Complex) superfamily, called Fucoxanthin-Chlorophyll *a/c*-binding
52 Proteins (FCPs), including three groups of proteins: Lhcf, Lhcr and Lhcx (Depauw et al. 2012;
53 Dong et al. 2016; Grouneva et al. 2011; ~~Depauw et al. 2012; Dong et al. 2016~~). With respect to
54 LHC, in FCPs chlorophyll *c* is located close to chlorophyll *a*, and lutein is replaced by
55 fucoxanthin. These characteristics enhance the capability of diatoms to harvest light in the
56 blue-green region (Premvardhan et al. 2010). Chrysolaminarin, a β -1,3-glucan, is the main
57 storage product, but also polyphosphates and lipids (long chain polyunsaturated and short
58 chain saturated fatty acids) can be accumulated (Round et al. 1990). A large vacuole is
59 present, which is responsible for floating upwelling and downwelling along the water column
60 (Raven 1987). Because of their siliceous frustule and their production of high quantities of
61 valuable molecules, diatoms gained importance for many biotechnological applications (see
62 Paragraph "The potential role of marine diatoms for biotechnological applications").

63 Diatoms are ubiquitous (Barragán et al. 2018; Kopalová et al. 2009; Malviya et al. 2016;
64 ~~Barragán et al. 2018~~). In aquatic ecosystems, they are distributed in almost all freshwaters,
65 seawaters (Malviya et al. 2016; Tsukazaki et al. 2018), and hypersaline waters (Clavero et al.
66 2000; Malviya et al. 2016; Round et al. 1990; ~~Clavero et al. 2000; Malviya et al. 2016~~). Diatoms
67 can be also epipelagic, epilithic, or epiphytic (Winter and Duthie 2000). Among marine diatoms,
68 the most studied ones belong to *Thalassiosira*, *Chaetoceros*, *Coscinodiscus*, *Skeletonema*,
69 *Phaeodactylum*, *Nitzschia*, *Cyclotella* genera.

70

71 **The potential role of marine diatoms for biotechnological applications**

72 Diatoms are the dominant component of phytoplankton, being responsible for up to 25% of
73 the global CO₂ fixation and contributing up to 40% of marine primary production (Field et al.
74 1998; Granum et al. 2005; Hildebrand et al. 2012). Characterized by complex evolutionary

75 history and recurrent genetic rearrangements, diatoms developed a unique metabolism and
76 subcellular organization, allowing them to greatly adapt to changes in environmental stress
77 conditions (Armbrust 2009).

78 The overall fast growth, high rates of CO₂ fixation, high photosynthetic efficiency and high
79 biomass and lipid productivities collocate diatoms among the best candidates for several
80 applications in different biotechnological fields. Food, pharmaceutical, bioremediation, bio-
81 energy and nanotechnology are the most common biotechnological applications of diatoms
82 ([Bozarth et al. 2009](#); [Delattre et al. 2016](#); [Fu et al. 2015](#); [Hildebrand et al. 2012](#); Lebeau and
83 Robert 2003a,b; [Bozarth et al. 2009](#); [Hildebrand et al. 2012](#); Levitan et al. 2014; [Fu et al. 2015](#);
84 [Mishra et al. 2017](#); Martinez Andrade et al. 2018; [Mishra et al. 2017](#)).

85 With the aim of making the microalgal large-scale production sustainable, it is necessary to
86 identify species that can grow in saltwater to avoid the competition with freshwater
87 resources (Hu et al. 2008; Popovich et al. 2012). In this perspective, marine diatoms may be
88 considered as an attractive feedstock for the production of a variety of bioactive compounds.

89

90 **Diatoms as sources of bioactive compounds and value-added products**

91

92 *Pigments*

93 Diatoms have been explored as sources of carotenoids (e.g. fucoxanthin and diadinoxanthin)
94 for application in food, feed, pharmaceutical and cosmeceutical industry ([Fu et al. 2015](#);
95 Lebeau and Robert 2003b; Spolaore et al. 2006; [Fu et al. 2015](#)). Fucoxanthin has received
96 much attention for its pharmaceutical role as antioxidant, anti-inflammatory and anticancer
97 molecule (Fu et al. 2015; Miyashita and Hosokawa 2018). Recently, Guo et al. (2016) have
98 screened 13 diatom strains for fucoxanthin accumulation and investigated the effect of

99 nutritional and environmental factors on the marine diatom *Cyclotella cryptica* as a promising
100 producer for fucoxanthin.

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102

103

104 *Medium-chain and long-chain polyunsaturated fatty acids*

105 Lipids are the major constituents of diatom cells. Their average lipid content could achieve up
106 to 25% of dry weight (dw) under normal growth conditions, although the production could be
107 enhanced under stress cultivation (Hildebrand et al. 2012; Levitan et al. 2014; Yi et al. 2017).

108 Concerning fatty acid (FA) profiles, diatoms are enriched with both medium-chain FAs and
109 very long chain polyunsaturated fatty acids (PUFAs) ([d'Ippolito et al. 2015](#); Pasquet et al.
110 2014; ~~d'Ippolito et al. 2015~~; Zulu et al. 2018). Diatoms are excellent accumulators of
111 eicosapentaenoic (EPA, 20:5n-3) and arachidonic acid (ARA, 20:4n-6). PUFAs are essential ω 3
112 fatty acids, mainly used as nutritional supplements in aquaculture (Lebeau and Robert 2003a;
113 Lopez et al. 2005; Merz and Main 2014), but also as additive in human food nutrition
114 (Spolaore et al. 2006). In particular, *Navicula saprophila* and *Phaeodactylum tricornutum* (2.2–
115 3.9% dw EPA), *Nitzschia inconspicia* (1.9–4.7% dw EPA) and *Nitzschia laevis* (2.5–2.76% dw
116 EPA) are cultivated for EPA production (Lebeau and Robert 2003b; Mishra et al. 2017 and
117 references therein).

118 Diatoms are also considered as a potential taxon for the biodiesel production ([Chen 2012](#);
119 [d'Ippolito et al. 2015](#); ~~Hu et al. 2008~~; ~~Chen 2012~~; Hildebrand et al. 2012; [Hu et al. 2008](#); [Joseph](#)
120 [et al. 2017](#); Levitan et al. 2014; ~~d'Ippolito et al. 2015~~; ~~Joseph et al. 2017~~; Zulu et al. 2018),
121 because they produce a large amount of triacylglycerides (TAGs), with percentages from 30 to
122 60% dw, with an average of 45% dw, under stress conditions ([Bozarth et al. 2009](#); [Chen 2012](#);
123 [d'Ippolito et al. 2015](#); [Hildebrand et al. 2012](#); Hu et al. 2008; ~~Bozarth et al. 2009~~; ~~Chen 2012~~;

124 ~~Hildebrand et al. 2012~~; Levitan et al. 2014; [Sabia et al. 2018](#); ~~d'Ippolito et al. 2015~~; Yi et al.
125 2017; ~~Sabia et al. 2019~~). TAGs can be converted into fatty acid methyl esters (FAMES), the best
126 substrate for biodiesel production.

128 *Other bioactive products and applications*

129 Microalgae produce a wide range of other bioactive compounds with antibiotic, antiviral, and
130 anticancer activity (Borowitzka 1995). Among these products we can mention: halogen-
131 containing compounds (Wichard and Pohnert 2006), extracellular polymeric substances ([EPS](#))
132 (~~Caldwell 2009~~[Delattre et al. 2016](#)), oxic domoic acid and isomers (Bates and Trainer 2006),
133 extracts with anti-tuberculosis activity (Lauritano et al. 2018), attractants and deterrents
134 (Frenkel et al. 2014), and long chain polyamines with biomineralization functions (Kröger et
135 al. 1999).

136 Researchers have succeeded in producing relevant polymers such as poly-3-hydroxybutyrate
137 (PHB), precursor of bioplastic in the marine diatom *P. tricornutum* (up to 10% dw), designing
138 diatoms as more desirable source of PHB for the production of biodegradable plastics
139 (Hempel et al. 2011).

140 Moreover, extensive researches have been focussed to explore the potential applications of
141 diatom frustules in nanotechnology (Lebeau and Robert 2003a; Kroth 2007; Ragni et al.
142 2017). Within this area, engineered biosensors (Bismuto et al. 2008), drug delivery systems
143 (Zhang et al. 2013), molecular filtration (Kroth 2007), solar cells, conductive electronic
144 devices (Jeffryes et al. 2011) and enzyme immobilizers (Poulsen et al. 2007) have been
145 examined.

147 **Diatoms as promising bioremediation agents**

148 Due to their high rates of CO₂ fixation and to their specific sensitivity to a variety of ecological
149 conditions, diatoms have been widely used as bioindicators ([Lobo et al. 2016](#); Reid et al. 1995;
150 Wang et al. 2008; ~~Lobo et al. 2016~~). In particular, ~~due to~~[thanks to](#) their high bioabsorption
151 ability, marine diatoms have been frequently used for the phytoremediation of heavy metals
152 contamination, in particular copper and cadmium (Lebeau and Robert 2003b; Monteiro et al.
153 2012; [Torres et al. 1998](#)). [Noteworthy is also that marine diatoms have also been proposed for](#)
154 [the elimination of emerging pollutants \(for example, ibuprofen or oxytetracycline\), as](#)
155 [reported by Santaefemia and co-workers \(2016, 2018\) for *P. tricornutum*.](#)
156 Among the various approaches for mitigating the global warming resulting from extensive CO₂
157 emissions due to human activities, the biological CO₂ biofixation has received much attention
158 as an alternative strategy to chemical reaction-based approaches ([Maity et al. 2014](#); Wang et
159 al. 2008; ~~Maity et al. 2014~~). In this perspective, marine diatoms may be considered as an
160 attractive feedstock for simultaneously combing CO₂ biomitigation coupled with biodiesel
161 production (Hildebrand et al. 2012; [Sabia et al. 2018](#); Wang et al. 2014; ~~Sabia et al. 2018~~).

162

163 **Genetic engineering for biotechnological and industrial applications of diatoms**

164 The available genome sequences of the centric diatom *Thalassiosira pseudonana* (Armbrust et
165 al. 2004) and the pennate *P. tricornutum* (Bowler et al. 2008) established these strains as
166 model marine diatoms for genomic, transcriptomic and metabolic studies (Poulsen et al. 2007;
167 Trentacoste et al. 2013). A recent review summarizes how, with the application of genetic
168 tools, it is possible to modify the natural metabolism of diatoms in order to favour the
169 production of new compounds with different biotechnological applications (Huang and
170 Daboussi 2017). Some studies reported that genetic modification could boost the neutral lipid
171 accumulation in marine diatoms (Armbrust et al. 2004; Trentacoste et al. 2013; Zulu et al.
172 2018). Moreover, genome editing of *P. tricornutum* (with TALEN and CRISPR/Cas9 - Daboussi

173 et al. 2014; Nymark et al. 2016) and *T. pseudonana* (with CRISPR/Cas9 - Hopes et al. 2016) has
174 been successfully demonstrated. The increase of fucoxanthin production in *P.*
175 *tricornutum* using genetic tools has been recently demonstrated (Perfeito et al. 2018).

176

177 **Cultivation methods of marine diatoms for biotechnological applications**

178 Recently, Maeda et al. (2018) showed the potential of marine microalgae (including brackish
179 and saline water microalgae) for biotechnological applications in large-scale industrial
180 production. Marine strains showed more resistance and tolerance to environmental changes,
181 as they are adapted to survive in high levels of salinity. Due to the large seawater availability,
182 the mass cultivation of halotolerant strains in brackish and seawater media can be considered
183 an interesting alternative to moderate the freshwater consumption (Popovich et al. 2012).
184 Moreover, in large-scale culture systems, the use the recycling culture medium has been
185 proposed as a possible solution (Sabia et al. 2015 and references therein) to lower the process
186 costs and to make microalgal production more environmentally and economically sustainable.
187 The efforts to enhance the production process of microalgae can be achieved by improving the
188 knowledge of the physiological metabolism of the microalgal cells, and through the
189 development of an effective and economic microalgal culture system in terms of engineering
190 and design aspects, within a biorefinery algal strategy ([Chen et al. 2011](#); Harun et al. 2010;
191 [Rizwan et al. 2018](#); ~~Chen et al. 2011~~; Wang and Seibert 2017; ~~Rizwan et al. 2018~~).

192 The growth performance and biochemical composition of a microalgal strain are significantly
193 dependent on culture conditions (Markou and Nerantzis 2013). The biochemical composition,
194 in fact, can be manipulated by altering the culture conditions and inducing an environmental
195 stress to increase and modify the accumulation of desired bio-compounds. As examples,
196 changes of the culture medium (nitrogen deficiency, organic carbon supply) as well as
197 changes in intensity (irradiance) and quality (spectral characteristics) of light can induce

198 modifications in photosynthetic efficiency, biomass production, cell morphology and fatty
199 acids storage (Chen 2012; [Baldisserotto et al. 2014, 2016](#); [del Pilar Sánchez-Saavedra et al.](#)
200 [2016](#); [Giovanardi et al. 2013](#); Markou and Nerantzis 2013; ~~Giovanardi et al. 2013~~;
201 ~~Baldisserotto et al. 2014, 2016~~; ~~del Pilar Sánchez Saavedra et al. 2016~~; Rizwan et
202 al. 2018; Sabia et al. 2018). Concerning marine diatoms, it is widely accepted that these micro-
203 organisms increase TAGs synthesis and accumulation under stress conditions, such as low
204 temperature and nitrate or silicate starvation ([d'Ippolito et al. 2015](#); [Hildebrand et al. 2012](#);
205 [Hu et al. 2008](#); [Levitan et al. 2014](#); ~~Hildebrand et al. 2012~~; ~~Hu et al. 2008~~; [Remmers et al. 2018](#);
206 Yu et al. 2009; ~~Hildebrand et al. 2012~~; ~~Levitan et al. 2014~~; ~~d'Ippolito et al. 2015~~; ~~Remmers et~~
207 ~~al. 2018~~) or under culture condition, such as mixotrophy (Liu et al. 2009; Wang et al. 2012) or
208 elevated CO₂ concentrations ([Sabia et al. 2018](#); Singh and Singh 2014; ~~Sabia et al. 2018~~).

209 The microalgal cultivation systems are crucial factors to be taken into account in order to
210 reduce the operative costs of microalgal production ([Borowitzka and Moheimani 2013](#); [Chen](#)
211 [et al. 2011](#); Rodolfi et al. 2009; ~~Chen et al. 2011~~; ~~Borowitzka and Moheimani 2013~~). A wide
212 variety of plant systems has been described in literature (Harun et al. 2010; Mata et al. 2010).
213 Marine diatoms have been cultivated in large-scale outdoor systems for aquaculture
214 ([Hildebrand et al. 2012](#); Lebeau and Robert 2003a,b; ~~Hildebrand et al. 2012~~), and recently for
215 biofuel production (Matsumoto et al. 2017; Wang and Seibert 2017). Recent progresses and
216 future perspectives in the commercial production of diatoms have been intensively reviewed
217 and presented by Wang and Seibert (2017).

218

219 **Light in aquatic environments**

220 Solar radiation is the driving energy that makes possible the production of organic matter *via*
221 photosynthesis by autotrophic organisms. The total solar radiation, which reaches the earth's

222 surface, extends in a spectral range from 300 (ultraviolet) to 4000 nm (infrared) and is
223 strongly reduced due to environmental, geographical, seasonal and physical parameters, such
224 as atmospheric scattering phenomena, weather conditions, latitude, altitude (Barsanti and
225 Gualtieri 2014; Ooms et al. 2016). Moreover, when light reaches the water surface and
226 penetrates the water column, it undergoes a further decrease. In particular, at the water
227 surface light is highly reflected and during water column penetration it undergoes attenuation
228 and narrowing processes of radiation bands. The latter process is due to absorption and
229 scattering events of water itself and materials dissolved therein (Kirk 1994). Red light and
230 infrared radiations are strongly, even if not completely, absorbed by water; this causes a
231 progressively enrichment in blue-green light as water deepness increases (Depauw et al.
232 2012; Mitra and Zaman 2016). This latter consideration is very important for all aquatic
233 photosynthetic organisms. In particular, the quality in spectral composition of
234 Photosynthetically Active Radiation (PAR), a constant fraction (400-700 nm) of solar
235 broadband irradiance (Pashiardis et al. 2017), and its quantity can influence growth of
236 aquatic plant organisms, which developed different strategies to make light use efficient
237 (Depauw et al. 2012; Schulze et al. 2014). Photosynthetic pigments for light capture and
238 management, in fact, have been differently evolved and organized inside the plastids in
239 different photosynthetic organisms, for example green and heterokont microalgae ([Depauw et](#)
240 [al. 2012](#); [Flori et al. 2017](#); [Lepetit et al. 2013](#); [Schulze et al. 2014](#); [Takaichi 2011](#); van den Hoek
241 et al. 1995; Wilhelm et al. 2006; ~~[Lepetit et al. 2013](#)~~; ~~[Takaichi 2011](#)~~; ~~[Depauw et al. 2012](#)~~;
242 ~~[Schulze et al. 2014](#)~~; ~~[Flori et al. 2017](#)~~). Diatoms synthesize carotenoids from β -carotene
243 pathway, and not from α -carotene, so they contain fucoxanthin (β -carotenoid) as their main
244 auxiliary pigment instead of lutein (α -carotenoid), which is the main auxiliary pigment in
245 green algae and plants ([Lavaud 2007](#); Wilhelm et al. 2006; ~~[Lavaud 2007](#)~~). This difference in
246 photosynthetic pigment composition is important in light harvesting at the plastid level, so

247 that chlorophytes (with chlorophyll *a* and *b*) better use red (ca. 630-680 nm) and blue (ca.
248 420-470 nm) light, while heterokontophytes and some dinoflagellates (with chlorophyll *c*)
249 better use blue light (Schulze et al. 2014 and references therein). Diatoms, which contain
250 fucoxanthin and chlorophyll *c* (Chl *c1*, *c2*, and/or *c3*), well harvest and use blue-green
251 radiations ([Lavaud 2007](#); Wilhelm et al. 2006; ~~Lavaud 2007~~).

252

253 **Light capture ability in diatoms**

254 In photosynthetic organisms, the action spectrum of light-harvesting pigments, which transfer
255 energy to the reaction centers of photosystems, and the absorption spectrum of incident light,
256 harvested by the cell influence the overall photosynthetic efficiency (Ooms et al. 2016). Like
257 other marine microalgae, diatoms can cope with strong fluctuations of light due to the fine
258 cellular and molecular mechanisms involved in the mediation of light responses (Brunet and
259 Lavaud 2010; Depauw et al. 2012 and references therein; Dong et al. 2016). In general, in
260 photosynthetic organisms and also in diatoms, upon short-term exposure to high irradiance
261 the photoprotection mechanisms are rapidly activated without changes in gene expression;
262 differently, if high light persists, gene expression is activated for the modulation of the
263 photosynthetic apparatus and of its photochemistry (Eberhard et al. 2008). Under low light
264 conditions, responses are usually slower than under high light and involve changes in the light
265 harvesting pigments in order to improve photocapture (Eberhard et al. 2008). In diatoms, a
266 highly active photoprotective mechanism is the non-photochemical quenching of chlorophyll
267 fluorescence (NPQ), which safely dissipates excess absorbed energy as heat (Ruban et al.
268 2004). Conversely, the “state transition”, as additional photoprotective mechanism for the
269 adjustment of energy distribution between PSII and PSI by phosphorylated LHCI complexes,
270 is not present ([Allen and Forsberg 2001](#); Owens 1986; ~~Allen and Forsberg 2001~~). An
271 exhaustive description of NPQ and other mechanisms involved in diatoms has been reviewed

272 by Depauw et al. (2012). Since NPQ is linked to carotenoid availability and to xanthophyll
273 cycle, it is clear that an accumulation of diatoxanthin is crucial to sustain this photoprotective
274 strategy under high light in diatoms, as in *T. pseudonana* (Zhu and Green 2010). Recently, the
275 diadinoxanthin/diatoxanthin pool is demonstrated to be important to strengthen the
276 antioxidant activity at the thylakoid membrane level under excess light conditions in *P.*
277 *tricornutum* (Lepetit et al. 2013). Moreover, specific fucoxanthin-chlorophyll *a/c*-binding
278 proteins (FCPs; Lhcx4, Lhcx6, Lhcr5 and Lhcr8) can bind to diatoxanthin under high light,
279 participating in photoprotection (Dong et al. 2016). Differently, other Lhcx proteins (Lhcx3
280 and Lhcx4) participate in NPQ regulation together to the diadinoxanthin/diathoxanthin
281 system in *P. tricornutum* under fluctuating light (Lepetit et al. 2017). Interestingly,
282 chlororespiration can play an important role in the regulation of photosynthesis with a
283 photoprotective meaning in marine diatoms, which often grow in turbulent waters, where
284 large fluctuations in light intensity occur (Lavaud et al. 2012). Chlororespiration is a process,
285 which allows the creation of a pH gradient over the thylakoid membrane in the dark, thus
286 promoting ATP synthesis (Kalaji et al. 2014). It is highly active in diatoms (Caron et al. 1987)
287 and can sustain xanthophyll cycle, leading to NPQ activity also in the dark (Jakob et al. 1999).

288 On the whole, responses to varying light (high, low, fluctuating) conditions in marine diatoms
289 involve a large variety of players and related metabolisms with protective and acclimative
290 roles: light harvesting complexes, photoreceptors and signaling proteins, ROS scavenging
291 systems, lipid and carbon metabolism, protein synthesis and even cell wall polysaccharides
292 modulations (Dong et al. 2016; Lepetit et al. 2017).

293 For light capture in underwater environments, as the marine one, the presence of blue light
294 sensors is crucial. Photoreceptors are proteins bound to a chromophore, an organic, non-
295 protein component that confers specific photochemical properties (Depauw et al. 2012;
296 Jaubert et al. 2017). Photoreceptors can participate in the regulation of the onset of cell

297 division, of the cellular rhythm, and of the dynamic behaviour of the photosynthetic apparatus
298 in many marine micro-organisms (Jaubert et al. 2017). Among different classes of
299 photoreceptors, diatoms contain two main blue light families, cryptochrome and
300 aureochrome (Depauw et al. 2012). The presence of red/far red sensors, which can bind to
301 biliverdin, has been recently discovered in *T. pseudonana* and *P. tricornutum* (Fortunato et al.
302 2016; Jaubert et al. 2017).

303

304 **Influence of light on diatoms: applicative point of view and examples**

305 Light intensity and its spectral characteristics are basic parameters to be taken into account
306 when considering microalgal growth with a biotechnological perspective. Even if sunlight is
307 cost-effective, the employment of artificial light can become economically advantageous and a
308 winning strategy if high-value microalgal biomasses are produced. It is, in fact, known that the
309 cultivation of microalgae under different growth conditions influences the biomass quality
310 (see Paragraph "Cultivation methods of marine diatoms for biotechnological applications"),
311 being light spectrum and intensity basic parameters that affect growth and cellular
312 composition of microalgae, marine diatoms included ([Orefice et al. 2016; Romero-Romero](#)
313 [and del Pilar Sánchez-Saavedra 2017](#); Schulze et al. 2014; ~~[Orefice et al. 2016; Romero-Romero](#)~~
314 ~~[and del Pilar Sánchez-Saavedra 2017](#)~~). If light is used to drive the biochemical composition of
315 microalgae, it is important to consider that light emitting diodes (LED) are a relatively new
316 and costless technology with respect to the traditional fluorescent light tubes, and offer the
317 great advantage to select nearly monochromatic lights at various wavelengths (Schulze et al.
318 2014). In the last few years, research in this context has increased.

319 The most characterising morphologic property of diatoms is the frustule, which can be
320 exploited in large biotechnological applications. Su and co-workers (2015, 2018) found that
321 variations in light intensity (100 to 300 $\mu\text{mol}_{\text{phot}} \text{m}^{-2} \text{s}^{-1}$) and spectrum (five monochromatic

322 LEDs: blue, green-yellow, red-orange, red, and a full visible spectrum white LED) are linked to
323 alteration in the features of the frustule of *Coscinodiscus granii* (valve thickness, Si
324 concentration, frustule diameter, foramen number, size and density). These alterations were
325 demonstrated to have an impact on the photonic properties of the frustule (Su et al. 2015). In
326 parallel, light wavelengths induced different growth rates: blue and red LEDs, together with
327 white LED, supported the best growth at both light intensities tested (Su et al. 2015). In a
328 recent work, it was found that EPA content in *P. tricornutum* under nitrogen starvation
329 conditions was influenced by light intensity, being 60 to 100 $\mu\text{mol}_{\text{phot}} \text{m}^{-2} \text{s}^{-1}$ the best ones able
330 to promote EPA accumulation with respect to higher intensities (250-750 $\mu\text{mol}_{\text{phot}} \text{m}^{-2} \text{s}^{-1}$)
331 (Remmers et al. 2018). More recently, Nur and co-workers (2018) studied the effect of both
332 light intensity and other environmental growth conditions (temperature, pH, nitrogen source,
333 use of palm oil mill effluent) on the productivity of fucoxanthin in the marine diatom *P.*
334 *tricornutum*, grown in nutrient-complete culture media, in the perspective of large-scale
335 outdoor cultivation. They observed that the fucoxanthin content was somehow inversely
336 proportional to light intensity. Moreover, in the same diatom the acclimation to high light
337 treatments was found to involve the activation of the xanthophyll-cycle photo-protective
338 mechanism without altering the fucoxanthin concentration (Fu et al. 2015 and references
339 therein). In *P. tricornutum*, blue light is considered essential in the photo-acclimation to high-
340 light intensities, but it also helps accumulation of carotenoids (Fu et al. 2015). On the other
341 hand, other works suggest that in the coastal diatom *Skeletonema marinoi*, blue light strongly
342 limits the photo-protective processes in the cells (Schellenberger Costa et al. 2012), probably
343 increasing the biochemical energy available for growth. Chandrasekaran and colleagues
344 (2014), moreover, exposed *S. marinoi* to a sinusoidal treatment of blue light with a peak at
345 midday and found that this light regimen was optimal for both growth and primary
346 metabolites production (lipids, carotenoids and proteins). In 2016, Orefice et al.

347 superimposed some fluctuating red light peaks to a sinusoidal treatment with blue light and
348 observed that NPQ and photo-protection mechanisms were enhanced by the presence of red
349 light, as already reported by Depauw et al. (2012). Parallel to different light responses of the
350 photosynthetic system in the presence/absence of red-light peaks, the biochemical
351 composition of the alga underwent alterations in terms of protein, carbohydrate and lipid
352 content. As regards carbohydrates, the Authors confirmed that providing only blue light
353 during algal cultivation is related to breakdown of carbohydrate reserves in the cells, as also
354 observed in the green microalga *Chlorella* (Kamiya and Saitoh 2002). On the contrary, lipid
355 content per cell was correlated to daily light dose, but not to the presence/absence of red-
356 light peaks. Interestingly, the lipid profile was affected by the different light treatments; in
357 fact, the presence of red light lowered the content of monounsaturated fatty acids (3-6%),
358 compared to that obtained from algae cultivated under only blue light (25%) (Orefice et al.
359 2016). Differently, red light seemed to promote protein synthesis with enrichment in
360 histidine, in accordance with results previously obtained in *P. tricornutum* (Jungadreas et al.
361 2014). This does not represent a surprise, since histidine plays multiple roles in protein
362 interactions and in enzymatic catalytic reactions (Liao et al. 2013). Finally, notwithstanding is
363 that, different from what normally occurs by applying white or solar light, high doses of blue
364 light do not stimulate lipid accumulation in *S. marinoi* (Orefice et al. 2016).

365

366 **Conclusion and perspectives**

367 Research on the application of different light regimes for the cultivation of marine diatoms
368 highlights the need to improve knowledge on this topic in the highest number of diatoms.
369 Manipulation of light in cultivation systems is, in fact, confirmed to be a key tool for increasing
370 microalgal productivity and quality, also for marine diatoms, but the response to light cannot
371 be easily generalised among photosynthetic micro-organisms. So, finding optimal light

372 conditions, specific for the cultivation of marine diatoms, could give them even a much more
373 importance as organisms for biotechnological applications.

374

375

376 **Aknowledgements**

377

378 This work was financially supported by the University of Ferrara, Italy

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