



HAL
open science

Current and novel approaches to downstream processing of microalgae: A review

Christos Nitsos, Rayen Filali, Behnam Taidi, Julien Lemaire

► To cite this version:

Christos Nitsos, Rayen Filali, Behnam Taidi, Julien Lemaire. Current and novel approaches to downstream processing of microalgae: A review. *Biotechnology Advances*, 2020, 45, pp.107650. 10.1016/j.biotechadv.2020.107650 . hal-03051736

HAL Id: hal-03051736

<https://hal.science/hal-03051736>

Submitted on 10 Dec 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Current and novel approaches to downstream processing of microalgae: A**
2 **review**

3 Christos Nitsos^a, Rayen Filali^a, Behnam Taidi^{b*}, Julien Lemaire^a

4 *^aLGPM, CentraleSupélec, Université paris-Saclay, SFR Condorcet FR CNRS 3417, Centre*
5 *Européen de Biotechnologie et de Bioéconomie (CEBB), 3 rue des Rouges Terres 51110,*
6 *Pomacle, France*

7 *^bLGPM, CentraleSupélec, Unierstiy of Paris Sacaly, Bât Gustave Eiffel, 3 rue Joliot Curie*
8 *91190 Gif-sur-Yvette FRANCE*

9 Christos Nitsos: christos.nitsos@centralesupelec.fr

10 Rayen Filali: rayen.filali@centralesupelec.fr

11 *Corresponding author Behnam Taidi: behnam.taidi@centralesupelec.fr

12 Julien Lemaire: julien.lemaire@centralesupelec.fr

13

14 **Current and novel approaches to downstream processing of microalgae: A**
15 **review**

16

17 Abstract

18 Biotechnological application of microalgae cultures at large scale has significant potential in
19 the various fields of biofuels, food and feed, cosmetic, pharmaceutical, environmental
20 remediation and water treatment. Despite this great potential application, industrialisation of
21 microalgae culture and valorisation is still faced with serious remaining challenges in culture
22 scale-up, harvesting and extraction of target molecules. This review presents a general summary
23 of current techniques for harvesting and extraction of biomolecules from microalgae, their
24 relative merits and potential for industrial application. The cell wall composition and its impact
25 on microalgae cell disruption is discussed. Additionally, more recent progress and promising
26 experimental methods and studies are summarised that would allow the reader to further
27 investigate the state of the art. A final survey of energetic assessments of the different
28 techniques is also made. Bead milling and high-pressure homogenisation seem to give clear
29 advantages in terms of target high value compounds extraction from microalgae, with enzyme
30 hydrolysis as a promising emerging technique. Future industrialisation of microalgae for high
31 scale biotechnological processing will require the establishment of universal comparison-
32 standards that would enable easy assessment of one technique against another.

33

34

35

36

37

38

39

40

41

42

43

44

45

46

47

48

49

50 Keywords: Biotechnology, microalgae, harvesting, downstream processing, cell wall
51 disruption, high value molecule extraction, bioenergy

	Page	
52	Index	8
53	1. Introduction	3
54	1.1 Microalgae in the context of biorefinery	5
55	1.2 Microalgal cell wall recalcitrance	6
56	2. Harvesting	8
57	2.1 Sedimentation	8
58	2.2 Coagulation-Flocculation	9
59	2.3 Centrifugation	11
60	2.4 Flotation	11
61	2.5 Membrane filtration	12
62	3. Pretreatment of microalgae & Extraction of target molecules	13
63	3.1 Physical methods	13
64	3.1.1 Milling	13
65	3.1.2 High-pressure homogenization (HPH)	15
66	3.1.3 Ultrasonication	17
67	3.1.4 Pulsed Electric Field	17
68	3.1.5 Drying	19
69	3.2 Chemical methods	19
70	3.2.1 Hydrothermal	19
71	3.2.2 Acid treatment	22
72	3.2.3 Alkaline treatment	23
73	3.2.4 Oxidative pretreatment	24
74	3.2.4.1 Ozonolysis	24
75	3.2.4.2 Hydrogen peroxide	25
76	3.3 Enzymatic methods	26
77	3.4 Novel downstream processing concepts	27
78	3.5 Energy efficiency of downstream processing	31
79	4. Conclusion and outlook	33
80	References	35
81		
82	1. Introduction	
83	Since CO ₂ has been identified as a potent greenhouse gas, reduction of anthropogenic CO ₂ emissions	
84	has become a focal point of both policy and research initiatives. The extended use of fossil hydrocarbon	
85	reserves to produce heat, electricity, transportation fuel and precursors for the chemical industry, have	
86	unbalanced the natural carbon cycle leading to increased CO ₂ levels in the atmosphere. This has been	

87 linked to the increase in the average global temperature. Several world agreements have promoted the
88 replacement of traditional fossil derived energy with renewable solar, wind, water, geothermal and
89 biomass alternatives (Chu et al., 2017; Demirbas et al., 2009). More specifically, the Paris Agreement
90 has set the ambitious goal to reduce CO₂ emissions to a level that will limit average global temperature
91 rise to less than 2 °C (Krug, 2018). In this context, not only reducing anthropogenic CO₂ generation, but
92 also CO₂ capturing technologies have become important.

93 Microalgae ability to fix CO₂ and convert it into useful molecules via photosynthesis makes them
94 attractive for the emerging bio economy. Microalgae can be divided into prokaryotic blue and green
95 algae (cyanobacteria), and eukaryotic microalgae that are further divided into *Chlorophyta*,
96 *Phaeophyta*, and *Chrysophyceae* based on the type of their photosynthetic pigments (green, brown and
97 gold, respectively) (Masojídek et al., 2013). The production of biomass and useful metabolic products
98 from a readily available and free carbon source is not the only advantage of microalgae. Their ability to
99 utilize inorganic nutrients such as nitrogen and phosphorus, allows their use for tertiary and quaternary
100 effluents treatment from secondary wastewater treatment process plants (Abdel-Raouf et al., 2012).
101 Organic carbon assimilation from microalgae in heterotrophic or photomixotrophic growth metabolism
102 is also possible, enhancing their wastewater treatment potential (Neilson and Lewin, 1974) and potential
103 valorization on the anaerobic co-digestion process. Coupling wastewater treatment with CO₂ capture
104 and biomass production may serve as an elegant and efficient solution of current environmental and
105 economic challenges, for example by integrating CO₂ from industrial flue gasses to microalgal
106 wastewater treatment (Molazadeh et al., 2019; Razzak et al., 2013). Although this type of an integrated
107 approach has not yet reached maturity, the All-gas project is an example of municipal wastewater
108 treatment, CO₂ fixation and biogas production that has reached the demonstration scale (Maga, 2017).

109 As with higher plants, microalgae require land for their production, although the use of coastal waters
110 are increasingly suggested. Microalgae, however, can give higher productivity compared to terrestrial
111 crops due to their continuous cultivation mode, fast growth rate, short cycle doubling time, year-round
112 operation and higher content in target molecules (Eing et al., 2013). By extrapolating results from
113 laboratory scale microalgae cultivation to the commercial scale it was estimated that several
114 *Chlorophyta* achieved much higher oil yields compared to land crops (Nascimento et al., 2014). For
115 example, *Chlamydomonas sp.* and *Chlorella vulgaris* oil yield was 10.4 m³ ha⁻¹ year⁻¹ and 44.4 m³ ha⁻¹
116 year⁻¹, respectively. Palm oil productivity on the other hand was estimated at only 6 m³ ha⁻¹ year⁻¹
117 (Nascimento et al., 2014). Biomass yields of microalgae is also higher and in the range of 70-100 t DW
118 ha⁻¹ year⁻¹ (metric tones DW per hectare per year), compared to 20 t DW ha⁻¹ year⁻¹ of miscanthus energy
119 culture (Eing et al., 2013; Lewandowski and Heinz, 2003), where DW is the dry weight of the sample.
120 Both open and closed systems have been used for microalgae culture. Open systems mainly consist of
121 raceway ponds with paddle wheels (Filali et al., 2019) and open cascade systems (Lee, 2001) and are
122 characterized by design and construction simplicity, lower capital cost, natural light and temperature
123 control and easier maintenance. Their disadvantages include microbial contamination, high area
124 demand, dependence on environmental conditions, insufficient mixing and mass transfer (Ugwu, C.U.
125 and Aoyagi, H., 2012; Acién et al., 2017; Eing et al., 2013). Closed culture systems (photobioreactors)
126 on the other hand require less space, enable better operational parameters control, allow axenic culture
127 conditions, and good culture mixing, but at higher construction and operation cost. This makes them
128 suitable for the cultivation of more microalgae species, including less robust species, or when the
129 targeted product purity is essential (Acién et al., 2017). Harvesting and concentration costs of microalgal
130 biomass is a major inconvenience of the overall biorefinery process in both reactor types.

131 Microalgae cultivation requires significant area and land availability can be an important limiting factor
132 of a microalgae-based bioeconomy. The low DW biomass productivity of photosynthetic microalgae
133 cultures means that large volume of water is required to produce relatively low biomass amounts.
134 Available alkaline or saline water reservoirs valorisation can partially cover the water requirements of
135 microalgae cultivation (Hannon et al., 2010); such a solution, however, would be limited to a small
136 number of microalgae species adapted to survive in harsh media. Industrial process water, with or
137 without supplements, can also serve as a growth medium for microalgae cultures.

138 Light is the main limiting factor in microalgae cultures (Carvalho et al., 2011). Photosynthetic biomass
139 growth and productivity relies on CO₂ assimilation, and the flux of light energy the cells are exposed to
140 (Clement-Larosiere et al., 2014). Additionally, exposure to light is not homogeneous inside
141 photobioreactors, and depends on the geometrical configuration and the culture systems hydrodynamic
142 properties. The cells auto-shading and formation of biofilms on the photobioreactor walls or on the
143 culture's surface also have a strong impact on light penetration. The drop in light intensity is very steep
144 even a few millimetres from the photobioreactor surface. Indeed, photobioreactors are naturally divided
145 into light and dark zones, with efficient photosynthetic activity occurring only in the light zones.
146 Depending on the bioreactor design parameters, such as tube diameter or pond depth, the dark areas can
147 constitute the majority of the reactor volume (Carvalho et al., 2011). Light limitation is a major reason
148 for the low productivity of photoautotrophic cultures compared to heterotrophic fermentations.
149 Temperature effects can also significantly affect production efficiency and may require temperature
150 control increasing investment and operation costs (Ras et al., 2013). Typical biomass concentration for
151 photoautotrophic cultures is in the region of 0.5 to 2.0 g L⁻¹, whereas in heterotrophic cultures biomass
152 concentrations of 18 g L⁻¹ (Cheng et al., 2009) and 24 g L⁻¹ (Chen and Walker, 2011) are possible. For
153 traditional industrial fermentations utilizing yeast, concentrations of greater than 50 or 60 g L⁻¹ can be
154 achieved (Di Serio et al., 2001; Paredes-López et al., 1976). In turn, the low cell densities of microalgae
155 cultures make downstream processing, such as cell concentration and extraction of valuable molecules,
156 critical to the success of industrial microalgae valorization (Gayen et al., 2019).

157

158 1.1 Microalgae in the context of biorefinery

159 Deriving value from microalgae through a biorefinery operation (Fig.1) can appear rather attractive as
160 the carbon nutrient (CO₂) is not only free but its removal from the atmosphere is desired. The use of
161 microalgae, however, presents several challenges: the growth of microalgae requires significant
162 quantities of carbon, nitrogen and phosphorus. The inorganic carbon required for photosynthesis can
163 come either from CO₂ or from dissolved bicarbonate ions in the medium (Gonçalves et al., 2017).
164 Inorganic nitrogen (nitrate, nitrite, ammonium, ammonia, molecular nitrogen) uptake is species
165 dependent (Hellebust and Ahmad, 1989). Organic compounds such as urea can also be used as a nitrogen
166 source (Kim et al., 2016). Phosphorus, on the other hand, is assimilated mainly as phosphate ions, with
167 the ability of organic phosphorus uptake also shown (Singh et al., 2018). Most microalgae have the
168 ability to uptake more phosphorus than is required for growth and store it in the form of inorganic
169 polyphosphate. This natural adaptation to phosphorus poor environments is termed luxury phosphorus
170 uptake (Solovchenko et al., 2019). The use of industrial fertilizers as sources of nitrogen and phosphorus
171 can be a cheaper alternative to growth medium formulation increasing the economic sustainability of
172 microalgae culture and valorisation. Magnesium (Mg²⁺), an important component of the photosynthetic
173 apparatus, is also critical for microalgae growth and biomass productivity is related to its concentration
174 in the growth medium (Ben Amor-Ben Ayed et al., 2016, 2015). Coupling microalgae biorefinery with
175 process water treatment and carbon dioxide biofixation is economically attractive for sustainable
176 microalgae culture. Process water is rich in inorganic content that is expensive to remove in traditional
177 tertiary process water treatment operations. Their use as growth media in a biorefinery would reduce the
178 cost of both process water treatment and microalgae culture. In addition, the process water would
179 provide a readily available source of water, and organic and/or inorganic compound necessary for
180 microalgae growth. The ability of microalgae to remove nitrogen (Delgadillo-Mirquez et al., 2016)
181 magnesium (Ben Ayed et al., 2017) and excess phosphorus -through luxury phosphorus uptake-
182 (Solovchenko et al., 2019) from their growth medium, makes them suitable for such water depollution
183 applications (Fig. 1). The biomass produced can be used as a fertilizer or converted to renewable fuels
184 and energy and increase the operation's sustainability. This could be an interesting solution despite the
185 problem of light penetration into the culture caused by the secondary effluent's turbidity and colour.
186 Lack or limitations of nutrients that are necessary for growth in the medium can affect the composition
187 of the microalgae biomass. Such limitations are often desirable and are induced as a stress factor; can
188 be accomplished by formulation of the growth medium. For example, nitrogen limitation in the latter
189 stages of microalgae growth can induce lipid production as an energy storage mechanism, and therefore

190 increase the value of the produced biomass (Juneja et al., 2013). The effects of limitation of major
191 nutrients in the growth medium can be seen in Table 1.

192 Figure 1

193 Table 1

194 The low dry weight content of microalgae cultures (typically around 1 g L⁻¹) makes harvesting and
195 concentration an expensive unit operation of any biorefinery. Harvesting and dewatering are the main
196 downstream processes required for any applications where the whole microalgae biomass can be used
197 directly as the final product, such as for animal and fish feed or food supplements (e.g. *Arthrospira*
198 *platensis* tablets and powder). Biomass concentration can be achieved by the combination of some
199 techniques such as settling, decantation, centrifugation and filtration; all followed by an operatory
200 process of microalgae conditioning by drying of the concentrated pastes (Raja et al., 2018). Harvesting
201 and dewatering processes may also include membrane filtration, shaking separators, flotation and
202 flocculation, or pressing.

203 The various molecules of interest in the microalgae biomass can be classified into structural e.g. cell
204 wall carbohydrates and proteins and cell membrane lipids; and non-structural e.g. storage lipids and
205 carbohydrates, vitamins, polyunsaturated fatty acids and pigments (Cuellar-Bermudez et al., 2015;
206 Shannon and Abu-Ghannam, 2018). These may be the final target molecules, as in the case of ω -3 or ω -
207 6 fatty acids and β -carotene that can be used in food supplements; or used as feedstock for fuels and
208 chemical products, as in the case of carbohydrates and lipids. Such diversity in the chemical composition
209 of microalgae, requires a fractionation step of the target molecule. The fractionation process must be
210 selective in order to maximize the desired molecule's yield and minimize formation of unwanted and
211 inhibitory products.

212 Cell disruption can be achieved by physical (Ultrasonication, High Pressure Homogenization, Bead
213 Milling, Pulsed Electric Field) chemical (Acid, Alkaline and Oxidation), thermal (Hydrothermal, Steam
214 Explosion) and biological methods (Enzymatic treatment) (Soo Youn Lee et al., 2017). During cell wall
215 disruption, intracellular molecules can be extracted with organic solvents (Araujo et al., 2013), ionic
216 liquids (Y. Zhang et al., 2018), supercritical CO₂ (Reyes et al., 2016; Cheng et al., 2011) or supercritical
217 mixtures of CO₂ and solvents (Obeid et al., 2018). Cell-wall components fractionation, may require their
218 further depolymerization via hydrothermal (Lorente et al., 2017), acid (Hernández et al., 2015) or
219 enzymatic hydrolysis (Sierra et al., 2017). The targeted molecules separation and purification can be
220 performed with chromatography (Bousquet et al., 1994; Fábryová et al., 2019) and membrane
221 technology (Giorno et al., 2013).

222

223 1. 2 Microalgal cell wall recalcitrance

224 The bulk production of fuels such as biogas (Córdova et al., 2018) or bio-oil through hydrothermal
225 liquefaction (Hu et al., 2017) are common methods of deriving value from microalgae biomass. In such
226 cases, cell disruption does not facilitate extraction or fractionation of molecules but enhances the
227 conversion reactions towards the desired end products (Mahdy et al., 2014a; B. Zhang et al., 2018). Cell
228 wall disruption may be essential for the efficient extraction of intracellular molecules such as pigments
229 and oils. Cell wall components may themselves be target molecules, requiring disruption for their
230 efficient fractionation e.g. carbohydrates for bioethanol production (Kim et al., 2012), or hydrocarbons
231 from the *Botryococcus* extracellular matrix (Ciudad et al., 2014)

232 Cell wall structure (Fig. 2) and composition has been extensively reviewed elsewhere (Baudeflet et al.,
233 2017). Cell wall thickness and chemical composition have been identified as the most important factors
234 determining the cell wall strength (Zhipeng Duan et al., 2017). Cell wall robustness can vary related to
235 microalgae species (Montsant et al., 2001). *Isochrysis Galbana* completely lacks a cell wall (Thronsdén,
236 1997), making it very fragile, whereas *Chlorella sp.* have a glucosamine rigid cell wall and a
237 hemicellulose-like cell wall layer mainly of galactose and mannose sugars (Rodrigues and da Silva Bon,

238 2011). *Haematococcus pluvialis* has a very thick cell wall composed of cellulose-like polysaccharides
239 and a very resistant polymer, algaenan (Hagen et al., 2002). Algaenan is one of the most inert microalgae
240 cell wall components and very resistant to physical, chemical and enzymatic pretreatment (Mendes-
241 Pinto et al., 2001).

242 The cell wall of the blue-green alga *A. platensis* is composed of four layers one of which is a more robust
243 peptidoglycan layer (Van Eykelenburg, 1977). *Tisochrysis lutea* has a cell wall consisting of two-layered
244 plates (Orlova et al., 2019). A comparative study of cell disruption of various species with
245 ultrasonication has correlated cell wall composition and structure to resistance to physical damage (shear
246 stress) (Zhipeng Duan et al., 2017). From the treatment of green algae and cyanobacteria the following
247 suggested order of cell wall robustness can be proposed for a few species: *Chlorella pyrenoidosa* >
248 *Synechococcus elongatus* > *Microcystis aeruginosa* > *Chlamydomonas reinhardtii*. The more resistant
249 cell wall of *C. pyrenoidosa* is composed mainly of cellulose rich polysaccharides, the cyanobacteria of
250 a peptidoglycan layer with an extracellular mucilage layer and a further, serrated external layer (S-layer)
251 and finally, of the rupture prone *C. reinhardtii* of hydroxyproline-rich glycoproteins (Zhipeng Duan et
252 al., 2017). The cell wall of *Chlorella* strains also contains a chitin-like layer (Kapaun and Reisser, 1995).
253 Besides the organic layers some microalgae cell walls have inorganic components, like the cell wall of
254 the diatom *Phaeodactylum tricornutum*, that contains a silica shell (Gügi et al., 2015).

255 Similar indirect suggestions for a possible correlation between cell wall composition and robustness
256 exist in other articles. In a study of rupture of *Tetraselmis suecica*., *Chlorella* sp. and *Nannochloropsis*
257 sp, by High Pressure Homogenization the pressure required to rupture 50% of the cells was determined
258 at 170 bar, 1060 bar and 1380 bar, respectively (Spiden et al., 2013). The order of cell wall robustness
259 of these species therefore can be proposed as: *Nannochloropsis* sp. > *Chlorella* sp. > *T. suecica*.
260 Although the cell wall composition of these species was not measured in the study, it is noted from the
261 literature that they are made up from polymers resistant to mechanical rupture, polysaccharides and
262 glycoproteins, and carbohydrate scales, for *Tetraselmis suecica*., *Chlorella* sp. and *Nannochloropsis* sp,
263 respectively (Spiden et al., 2013). In a similar approach, cell fragility at 1750 bar was studied (Taleb A.
264 2016) from which the order of cell wall robustness can be proposed for the following species:
265 *Scenedesmus* sp. UTEX 1589 > *Nannochloropsis salina* 537 CCMP > *Scenedesmus obliquus* 393 UTEX
266 > *Nannochloropsis gaditana* 527 CCMP > *Parachlorella kessleri* 2229 UTEX. Again, the cell wall
267 composition of the species was not provided.

268 A second level of recalcitrance is presented by microalgae organized in coenobial communities, held
269 together by extracellular structures. For example, *Botryococcus* strains have thick cell walls, rich in
270 hemicellulose-like carbohydrates predominantly of arabinose and galactose composition, an algaenan
271 layer, and form colonies held together with a hydrocarbon extracellular matrix. Equally complex is the
272 cell wall of *Scenedesmus*, containing cellulose-like carbohydrate layers, algaenan and an external
273 coenobial sheath when the cells are grown in colonies.

274

Figure 2

275 Modifications during the different stages of microalgae growth can also significantly affect the
276 recalcitrance of the cell wall. For example, *H. pluvialis* vegetative cells are susceptible to breakage at
277 moderate pressures (4000 to 10,000 psi), whereas cysts with thicker algaenan-rich cell walls required
278 very high pressure (20,000 psi) for complete cell disruption (Montsant et al., 2001). It has also been
279 shown that growth rate can also affect cell wall robustness, as cultures grown at higher rates produce
280 cells with thinner walls (Němcová and Kalina, 2000). In general, the thickness and chemical
281 composition of the cell wall can vary a lot even in the same species depending on the growth conditions
282 (Liu et al., 2006). Concerning the enzymatic methods, the multilayered structure of a cell wall poses an
283 additional challenge as each layer may require enzymatic treatment by a different class of enzymes,
284 increasing the enzymatic cocktail's complexity and cost, or requiring multi-stage pretreatment. In
285 electricity-based pretreatment methods, such as Pulsed Electric Field, the small size of the microalgae
286 cell also enhances resistance to disruption.

287 Structural modifications induced from the specie's growth state, energy requirements, pretreatment costs
288 and price of the desired products must be carefully considered in order to select an appropriate cell

289 disruption method. Sensitivity of the targeted molecules to degradation during pretreatment must also
290 be considered in order to minimize losses in the final product yield and biological activity. Some
291 interesting microalgae species, their cell wall composition, targeted molecules, and their respective
292 prices, are summarized in Table 2.

293 Table 2

294

295 2. Harvesting

296 Harvesting is the solid-liquid separation of cells from the growth medium. The microalgae cells are the
297 main culture product, whereas the nutrient depleted medium is a valuable water source that can be
298 recycled into the bioreactor directly or supplemented with nutrients, if required. The very low
299 microalgae biomass concentrations make harvesting a critical operation unit for a biorefinery from the
300 economical point of view. Further downstream processing steps such as cell disruption and extraction-
301 fractionation cannot be performed at low solids content as the high diluted solutions would not be
302 treated. The typically low biomass yield in microalgae production systems poses severe economic and
303 energetic restrictions to the harvesting and consequently to the whole biorefinery process. Operating
304 cost cannot exceed the value of final products, and since at least some of the biorefinery end products
305 will be fuels, the harvesting energy should not exceed the biomass energy content. The relatively small
306 size of microalgae cells -typically between 2 and 25 μm as well as the negative surface charge pose
307 additional challenges to the harvesting step. Harvesting can be divided into preconcentration, aiming to
308 increase the initial biomass content from 0.5-1.0% to around 3% and dewatering that can lead to a very
309 concentrated microalgal biomass, up to 25% in dry weight (Muylaert et al., 2017). In the case of larger
310 size filamentous microalgae like *A. platensis* the biomass can be efficiently harvested using vibrating
311 sieves, a relatively simple technology (Shelef et al., 1984).

312

313 2.1 Sedimentation

314 Natural sedimentation of microalgae cells is a very simple and low-cost harvesting method. The speed
315 of sedimentation depends on cell diameter and the density difference between the cell and the growth
316 medium, according to Stoke's equation, commonly used as a first approximation:

$$317 V_s = \frac{gd^2(\rho_c - \rho_m)}{18\mu}$$

318 where, V_s is sedimentation velocity, g acceleration of gravity, d cell diameter, ρ_c microalgae cells density,
319 ρ_m medium density, and μ the medium viscosity. The Stoke's equation can describe sedimentation of
320 round shaped cells with good approximation, however its use is not universal and for different cell
321 shapes the equation cannot be applied (Peperzak et al. 2003). Indeed, when the shape of the microalgae
322 is not spherical or where there are formations like thorns on the surface of the cell the correlation of cell
323 size to settling rate can be negative (Peperzak et al. 2003). A negative correlation between size and
324 sedimentation rate has also been observed in the case of some microalgae colonies (Peperzak et al.
325 2003). It seems that cell density -or the difference between cell and medium density- is more significant
326 and higher cell densities will result in higher sedimentation rates (Peperzak et al. 2003). Sedimentation
327 velocity can vary several orders of magnitude among microalgae species depending on cell size, density
328 and shape. As a general rule small diameter and low-density microalgae tend to sediment very slowly
329 compared to larger and denser cells (Mathimani and Mallick, 2018).

330 In mixed microalgae cultures, sedimentation by gravity is an efficient harvesting method if the colony
331 is dominated by species with large settling velocities. When slow settling microalgae become dominant
332 the harvesting efficiency is reduced even at prolonged settling periods of 24h (Park et al., 2011). Natural
333 sedimentation has been proposed as a harvesting method or pre-concentration step even for slow settling
334 microalgae like *Monoraphidium* sp. that could be harvested after 24 h with a 98% yield (Yu et al., 2012).

335 The filamentous cyanobacterium *A. platensis* with a fast sedimentation velocity of 0.64 m h^{-1} and
336 accumulation of carbohydrates during nitrogen starvation could be concentrated 15 times by
337 spontaneous sedimentation that achieved 93% removal of water (Depraetere et al., 2015). The diatom
338 *Amphora* with the impressive sedimentation velocity of 2.91 m h^{-1} , can be harvested by a simple low
339 cost and fast gravity sedimentation (Chtourou et al., 2015). It also exhibits relatively fast growth rate
340 and significant fat accumulation. Microalgae with such properties have a great advantage compared to
341 slowly settling ones in terms of harvesting efficiency. Their valorization could be prioritized over other
342 species as they may be an economically viable solution to a truly efficient microalgae biorefinery.

343 Nitrogen starvation in *Nannochloropsis salina* and *Chlorella* sp. cultures led to reduction in the cell
344 density and settling velocity by as much as an order of magnitude at the latter culture stages (Baroni et
345 al., 2019). This could be a disadvantage for harvesting as nitrogen starvation is typically related to lipid
346 accumulation, and the lipid rich cells could be harder to separate. A compromise might be necessary
347 between biomass, lipid yields and settling velocity if unassisted gravity sedimentation is being
348 considered as the sole method of harvesting. However, as gravity sedimentation can seldom induce the
349 required biomass dewatering, its use for pre-concentration followed by another method is preferable.
350 For example, gravity sedimentation coupled with centrifugation can reduce the harvesting cost and
351 energy by a factor of 30 compared to centrifugation alone (Badvipour et al., 2016).

352

353 2.2 Coagulation-Flocculation

354 Coagulation and flocculation are conventional technologies used in water treatment to promote the
355 separation of small particles by sedimentation. Both methods involve the addition of compounds which
356 cause the agglomeration of particles into larger flocs to increase their sedimentation velocity, according
357 to Stoke's equation. Coagulation is a chemical technique based on the neutralization of charge of the
358 microalgae surface to disrupt the repulsive forces and promote cell aggregation. Flocculation is a
359 physical method that uses compounds on which cells can clump together without involving surface
360 charge neutralization. There is a subtle difference between the two phenomena but in the scientific
361 literature most authors only refer to flocculation.

362 Coagulation can occur with the addition of inexpensive multivalent salts such as $\text{Al}_2(\text{SO}_4)_3$ that could
363 remove more than 85% of *N. salina* in 62 min, although at a relatively high dosage - 229 mg L^{-1}
364 (Chatsungnoen and Chisti, 2016). Coagulation of *C. reinhardtii* at elevated pH values was induced by
365 ferric (FeCl_3), calcium (CaCl_2), and magnesium chlorides (MgCl_2). Even at very low concentrations (>5
366 mM) more than 90% biomass harvesting could be achieved making this an attractive and inexpensive
367 method (Fan et al., 2017).

368 Coagulation with multivalent cations can be improved by pH adjustment to basic values. Indeed, its
369 efficiency can be related to the cells zeta potential, a pH depended phenomenon (Fan et al., 2017).
370 Adjustment of pH to 9.51 induced coagulation of *Chaetoceros calcitrans* and 89% of cells were
371 harvested with a sedimentation rate of 0.125 m h^{-1} and a concentration factor of 4 in 10 min (Şirin et al.,
372 2015). Adjustment of pH to 10.2 with addition of sodium or potassium hydroxide led to high harvesting
373 efficiency ($> 90\%$) of *C. calcitrans* biomass. At increased polyelectrolyte dosages the sedimentation
374 rate was greatly improved without loss of cell viability or harvesting yield (Harith et al., 2009). *C.*
375 *vulgaris* biomass required a pH higher than 11 for coagulation efficiency above 90% with magnesium
376 hydroxide for reuse (Vandamme et al., 2012). Microalgae biomass contamination by the coagulation
377 reagents is the biggest process disadvantage as they can reduce the extracted biomolecules quality or
378 interfere with other downstream processing operations (Vandamme et al., 2012). This can be overcome
379 by re-suspending the coagulation reagent after harvesting. For example in the coagulation of *C. vulgaris*
380 and *Phaeodactylum tricorutum* with $\text{Mg}(\text{OH})_2$, a mild acidification of the microalgae slurry could
381 dissolve 95% of the precipitated magnesium hydroxide (Vandamme et al., 2015).

382 The use of inorganic reagents can be less effective when used for the harvesting of marine microalgae
383 due to the shielding of cells by ions in the medium that lead to inhibition of floc formation (Vandamme
384 et al., 2013). Organic polymers on the other hand can be effective flocculants for both marine and

385 freshwater microalgae (Table 3). Organic polymers with positive charges also neutralize the negative
386 surface charge of microalgae cells but can act on several cells simultaneously leading to a bridging effect
387 (Figure 3) and floc formation. Reduced harvesting efficiencies have sometimes been observed at
388 increased dosages of cationic polymers (Lam, 2017). This has been explained by a protection of the cells
389 by the excess flocculant leading to surface charge reversal and repulsive forces from the cationic
390 polymers inhibiting floc formation (Figure 3).

391 Figure 3

392 To achieve flocculation, charged organic polymers can be used such as, chitosan a natural-based cationic
393 biopolymer, which is efficient at low concentrations. At 20 mg L⁻¹ and pH 8 a 90% harvesting efficiency
394 and 80% cell viability of *C. calcitrans* is possible (Harith et al., 2009). Although alginate has been shown
395 to not induce flocculation in some cases (Zhu et al., 2014), its addition with chitosan that has an opposite
396 charge, creates a polyelectrolyte complex that can enable harvesting of microalgae by flocculation at
397 ppm range concentrations. *Scenedesmus obliquus* biomass was harvested with an efficiency of 86%,
398 with this way (Matter et al., 2018). Cationic starch is another cationic biopolymer that can induce
399 flocculation of *Chlorella protothecoides* at 40 mg L⁻¹ and achieves an extraction efficiency of 98% at
400 near neutral and alkaline pH values (Letelier-Gordo et al., 2014). Polyacrylamide is another type of
401 organic polymer that has been extensively used for harvesting of microalgae by flocculation. They
402 achieved very high flocculation efficiencies between 90% and 100% for a variety of microalgae species.
403 (Van Haver and Nayar, 2017). The charge of the polymers seems to play a more important role in the
404 case of marine microalgae. For example, in the marine microalgae *N. salina* using polyacrylamide
405 polymers of medium, high, and very high charge -with all other parameters kept the same- improved the
406 harvesting efficiency from 73% to 88% to 94%, respectively (Table 3). This effect was not observed for
407 the freshwater microalgae *C. vulgaris*, where the harvesting efficiency remained almost 100%
408 irrespectively of the charge of the polymer (Van Haver and Nayar, 2017). This could be caused by the
409 presence of salt ions in the marine microalgae medium that can partially neutralize or counteract the
410 charge of the polymer; or it could be related to the surface charge of the individual species. In both cases
411 it is worth noting that the very promising harvesting results were achieved with very low polyacrylamide
412 dosages; 3 mg L⁻¹ for *N. salina* and 1.66 mg L⁻¹ for *C. vulgaris* (Van Haver and Nayar, 2017). Despite
413 these promising results, polyacrylamides are potentially hazardous chemicals and their presence can
414 contaminate the harvested biomass, making it unsuitable for pharmaceutical, cosmetic and food
415 applications, and limiting their use to biofuels production.

416 Tannins is a class of phenolic biomolecules -present in plant biomass- that are very efficient flocculants
417 (Table 3). POLYSEPAR®CFL25, a tannin quaternary ammonia salt with low molecular weight and high
418 charge showed 95 % harvesting efficiency of *Chlorella sp.* cells at a dosage of 30 mg L⁻¹ (Van Haver
419 and Nayar, 2017). Tanfloc SL, another tannin polymer of low molecular weight and low to medium
420 charge could harvest 100% of *C. vulgaris* cells at a dose of 5 mg L⁻¹ (Van Haver and Nayar, 2017). A
421 lower cost bio flocculant has been produced by fermentation of rice bran with *Bacillus agaradhaerens*
422 C9. The bioflocculant is possibly a hemicellulose oligomers hydrolysate as xylanase activity was
423 detected in the fermentation broth. It achieved around 90% harvesting efficiency at a 60 mg L⁻¹
424 concentration (Liu et al., 2017). Other bioflocculant polymers such as poly γ -glutamic acid produced by
425 *Bacillus Licheniformis* have been successfully used for the flocculation of *Desmodesmus brasiliensis* at
426 > 98 % efficiency. Anionic and non-ionic polymers showed no or poor flocculation efficiency in most
427 cases, or had inferior performance compared to cationic ones (Tilton et al., 1972; Udom et al., 2013;
428 Uduman et al., 2010). This could be caused by the fact that the surface charges of microalgae are usually
429 negative (Danquah et al., 2009) and their neutralization requires positively charged flocculants or
430 combination with coagulants. Another interesting approach is thermal flocculation without the addition
431 of a flocculating agent. For example, thermal treatment of *C. vulgaris* and *S. obliquus* induced the
432 secretion of organic matter that acts as a flocculating agent and causes the formation of flocs. The authors
433 have hypothesized that the organic matter can be polysaccharides and/or protein (Xue et al., 2019)

434 As harvesting can represent up to 30% of the total microalgae processing cost, coagulant or flocculant
435 price can have a negative impact on biorefinery economics (Wu et al., 2015). Prices can vary
436 significantly between the less expensive coagulant salts such as Al₂(SO₄)₃ (0.2 \$ kg⁻¹) and the more

437 expensive flocculant: chitosan (19.6 \$ kg⁻¹) (Wu et al., 2015). It has been deduced that coagulation-
438 flocculation can be only marginally cheaper than centrifugation; auto flocculation, bioflocculation and
439 electroflocculation methods were suggested as more economically viable alternatives (Vandamme et al.,
440 2013). The use of polyelectrolytes as economical flocculants has also been proposed, as the doses
441 required are up to two orders of magnitude less than salts or chitosan and their cost is comparable to the
442 inexpensive salt coagulants. (Granados et al., 2012).

443

444 Table 3

445

446 2.3 Centrifugation

447 Centrifugation process simply increases the g force accelerating settlement as stated by Stokes equation.
448 Strain morphology can play an important role in the centrifugation efficiency. For example, linear *A.*
449 *platensis* filaments could be harvested at around 85% efficiency at 4000 x g for 10 min, in a laboratory-
450 scale fixed angle rotor centrifuge. In contrast helical *A. platensis* filaments showed poor harvesting yield
451 (around 50%) even at 9000 x g (Cuellar-Bermudez et al., 2019). *S. obliquus* cells could be removed from
452 the culture by centrifugation at 3000 x g for 10 min, in a 750W TD5A centrifuge, with a harvest
453 efficiency of 99.3% (Wang et al., 2019). Various types of centrifuges can be used to harvest and
454 concentrate microalgae, such as, spiral plate centrifuge (Collet et al., 2011), decanter centrifuge
455 (Adesanya et al., 2014), disk stack centrifuge (Milledge and Heaven, 2011), and hydrocyclone (Amaro
456 et al., 2017).

457 Although centrifugation is a very efficient dewatering method, it has some of the highest energy
458 requirements (Guldhe et al., 2016). Especially when microalgae are used to produce biofuels, the overall
459 energy consumption should ideally be lower than the biofuel energy content. When producing biogas
460 from microalgae, it was shown that neither centrifugation nor flocculation or sedimentation as a
461 standalone harvesting method can meet this criterion as the energy input was greater than the energy
462 content of the produced biogas. On the other hand, a positive energy balance can be achieved when
463 centrifugation is combined with pre-concentration by sedimentation or flocculation, (Milledge and
464 Heaven, 2017). Flocculation is used to preconcentrate the microalgal biomass or to reduce the energy
465 costs of harvesting by producing large size flocs that can be centrifuged more efficiently than single
466 cells (Collotta et al., 2017; Fan et al., 2017). In the harvesting of *Chlorella sorokiniana* cells by
467 centrifugation, with or without preflocculation, the benefits of preflocculation were clearly shown.
468 Preflocculation of the cells was performed with a dosage of 10 mg chitosan g⁻¹ dry algae weight more
469 than 99% clarification efficiency. The performance of centrifugation with and without flocculation was
470 similar (Xu et al., 2013). However, preflocculation decreased the volume of the algae suspension
471 between 20 and 50 times, with more than 95% of the water being removed in the latter case, leading to
472 significant reduction in the energy for dewatering (Xu et al., 2013). Preconcentration by natural settling
473 can be another option for energy reduction of centrifugation. It was estimated, for example that the
474 energy consumption for concentration of *C. vulgaris* cells by spiral plate centrifugation could be as low
475 as 0.042 kWh kg⁻¹ of biomass if preconcentrated by natural settling (Collet et al., 2011).

476 Disc stack centrifuges, on the other hand have a much higher energy consumption (Milledge and
477 Heaven, 2011) that can represent almost up to half of the total energy requirements of a biorefinery
478 (Mata et al., 2014). Hydrocyclone centrifugation is a low-cost and low energy option for harvesting
479 microalgae cells, however, as this method can achieve low cell concentrations (below 0.4% suspended
480 solids) its usefulness is limited to preconcentration (Amaro et al., 2017). Decanter centrifuges can
481 achieve high solid concentrations (approx. 20% DW) in continuous mode with pulsed discharges of the
482 concentrated biomass. Its use is suggested together with flocculation (Ramos Tercero et al., 2014).

483

484 2.4 Flotation

485 Flotation can also be considered a gravity based harvesting method, but the strategy is opposite to the
486 processes discussed above. The aim of this technique is to transfer the microalgae from suspension to
487 the medium surface where they can be easily harvested by skimming. This is achieved by the introducing
488 gas bubbles that attach to the cells and lift them. The microalgae cell attached to the gas bubble has
489 much lower density compared to the medium. According to Stoke's equation, this creates a negative
490 settling velocity or a rising velocity.

491 Several variations of flotation harvesting exist. In dissolved air flotation (DAF), pressure reduction of
492 air saturated water leads to bubble formation. When optimized it can lead to high biomass harvesting
493 yields. A 91% harvesting efficiency was achieved with dissolved air flotation in the pilot scale with a
494 floatation tank of 3.8 L (Niaghi et al., 2015). DAF is usually more expensive due to the cost of water
495 pressurization. In dispersed air flotation, air bubbles are created by gas injection into the medium
496 through a diffuser or sparger, often assisted by mechanical agitation for more efficient dispersion.
497 Bubble sizes typically fall within the 60 to 655 μm range with a concentration of $2 \cdot 10^5$ bubbles cm^{-3} .
498 The process efficiency depends on the formed bubbles stability as well as the proper bubble size
499 (optimally below 500 μm) (Alhattab and Brooks, 2017). Dispersed air flotation has been used to harvest
500 *Chlorella saccharophila* (Alhattab et al., 2019). Flotation is often combined with flocculation to enable
501 harvesting of concentrated flocs. This is an efficient harvesting method with reported harvesting yields
502 of 93.6 % for *S. obliquus* by thermal flocculation and air flotation (Xue et al., 2019); or 80% harvesting
503 of *Dunaliella salina* by combining NaOH-induced coagulation and DAF in the pre-industrial scale
504 (Besson et al., 2019). Flotation has also been combined with bioflocculation either by inducing
505 exopolysaccharides production in *A. plantensis* with more than 90% harvesting efficiency (Vergnes et
506 al., 2019); or bioflocculant produced from *Cobetia marina* for the flocculation-flotation of *C. vulgaris*
507 also with higher than 90% efficiency (Lei et al., 2015).

508 Besides air, flotation can also be achieved by other gases such as ozone. The harvesting efficiency
509 reported for an ozone dosage of 0.23 mg mg^{-1} of dried biomass was 79.6%. Although not so efficient,
510 ozonoflotation simultaneously pretreats the microalgae cells and increases the lipid extraction yields
511 (Velasquez-Orta et al., 2014). Ozone reduces the microalgae carbohydrate and protein content due to
512 cell lysis and partial release of these molecules in the medium. Denaturation of these proteins leads to
513 foaming that acts as a surfactant and aids flotation (Nava Bravo et al., 2019). Indeed, the addition of
514 surfactants like cetyl trimethylammonium bromide (CTAB), has verified the beneficial effect of foaming
515 on flotation. Foam facilitates microalgae cell attachment to the air bubbles by modifying the
516 hydrophobicity of the bubble surface. The surfactant's introduction could modify the extracted lipids
517 chemical composition, possibly due to increased extraction of lipids from the cell membrane's lipid
518 bilayer (Coward et al., 2014). The heat-induced flotation of *Scenedesmus dimorphus* at 85 °C has been
519 proposed as a method for the utilization of heat from off-gases of industrial processes, with harvesting
520 efficiency around 80% (Laamanen and Scott, 2017).

521

522 2.5 Membrane filtration

523 Membrane filtration, batch or continuous, is a very common harvesting method that can be employed
524 as dead-end with vertical flow across the membrane, or as tangential filtration where the flow is parallel
525 to the membrane surface (Hung and Liu, 2018). Membrane material can be ceramic (Jana et al., 2018) or
526 polymer (Zhao et al., 2016), and the process can be microfiltration (Kim et al., 2019) or ultrafiltration
527 (Zhang et al., 2019), with membrane pore sizes 10 μm - 0.1 μm and 0.1 μm - 0.01 μm , respectively.
528 Molecule retention rate depends not only on membrane pore size but also on their chemical nature
529 (polymeric, ceramic etc.).

530 Nylon membrane filtration is a very common filtration method. Gravity-driven dead-end filtration
531 through 5 μm nylon membranes was enough to achieve > 90% harvesting efficiency of *A. platensis*
532 cultures. Pumped filtration additionally increased the filtration flux up to $20 \text{ m}^3 \text{ m}^{-2} \text{ h}^{-1}$ enabling a faster
533 process (Cuellar-Bermudez et al., 2019). The flux of typical membrane filtration processes can be
534 considerably smaller. For example, in the harvesting of *Arthrospira* sp. with ceramic microfiltration and

535 ultrafiltration membranes, fluxes of 230 L m⁻² h⁻¹ and 93 L m⁻² h⁻¹ were reported, respectively (Jana et
536 al., 2018).

537 For marine microalgae, growth medium salinity can have detrimental effects on harvesting efficiency
538 by tangential-flow membrane filtration. Small increases in salinity of *Picochlorum* sp., and *Tetraselmis*
539 sp. growth medium significantly increased membrane fouling and reduced the permeate flux and
540 microalgae concentration factor, at the same time increasing the process energy requirements (Das et
541 al., 2019). Membrane filtration performance is also influenced by the culture's growth phase. For
542 example, harvesting of *Scenedesmus acuminatus* at the latter growth stages through ultrafiltration
543 membranes increased flux from 97 L m⁻²h⁻¹, to 131 L m⁻²h⁻¹ leading to faster processing by 36 %. This
544 was caused by the reduction of the average molecular weight of algogenic organic matter (AOM)
545 secreted by the cells in the latter stages. The high molecular weight content of AOM (higher than 50
546 kDa) decreased from 50% in the exponential phase to 42% in the stationary and 26% in the declining
547 phase. As the high molecular weight fraction of AOM has the greatest membrane fouling potential its
548 reduction led to minimized membrane fouling and increased flux. However, algogenic organic matter
549 negatively impacted the process efficiency through enhanced fouling at all stages of ultrafiltration (Ye
550 et al., 2019). Fouling is the major problem of membrane harvesting as it can negatively impact the
551 process performance, reducing flux and increasing energy requirements for efficient harvesting (Elcik
552 and Cakmakci, 2017). Regarding the effect of different types of AOM on fouling, it was found that
553 polysaccharides excreted by the microalgae cells in the medium were related to reversible membrane
554 fouling, whereas deposition of excreted protein led to irreversible membrane fouling (Jiang et al., 2018).
555 Other soluble microbial metabolism products such as humin-like substances also contribute to
556 irreversible membrane fouling (Wu et al., 2018; Zhao et al., 2017).

557 Fouling can be dealt with by adding surfactants like CTAB that remove hydrophilic and high molecular
558 weight foulants and additionally lead to electrostatic microalgae cells neutralization allowing for more
559 efficient dewatering (Taghavijeloudar et al., 2019). Alternatively, it can be prevented by the designing
560 of antifouling membranes (Elcik et al., 2017), e.g. containing novel materials such as carbon nanotubes
561 that improve membrane hydrophilicity (Khairuddin et al., 2019). Another approach is the introduction
562 of local turbulence. This was achieved by a membrane module with a cylinder with holes at its center
563 that created turbulent jets ejecting the feed into the membrane surface in a perpendicular direction. When
564 used for the microfiltration of *C. vulgaris*, at a speed of 7 L min⁻¹ fouling was reduced by 126%
565 compared to conventional type modules such as hollow fiber membrane (Kim et al., 2019). Application
566 of a shearing vibration is also able to reduce both reversible and irreversible fouling (Zhao et al., 2018,
567 2017). Cake layer formation of the filtered microalgae cells also contributes to fouling and increased
568 membrane resistance (Wu et al., 2018). Introduction of rigid particles both in dead-end and crossflow
569 filtration, as in the case of *Chlorella* sp. harvesting, can reduce the cake resistance and increase the flux
570 and the membrane performance (Hung and Liu, 2018). Introduction of flocculants during the ceramic
571 membrane filtration (both micro- and ultra-) of *Arthrospira* sp. also reduced fouling. This is caused as
572 flocculation leads to the formation of larger size particles and reduces their ability to enter the membrane
573 matrix (Jana et al., 2018). Indeed, membrane filtration is often used in combination with flocculation
574 due to the synergy of the two methods (Sahoo et al., 2017; Shi et al., 2017).

575 Table 4

576

577 3. Pretreatment of microalgae & Extraction of target molecules

578 3.1 Physical methods

579 3.1.1 Milling

580 Milling is one of the most promising methods for cell disruption of microalgae. Although it has been
581 studied in various setups and sizes such as lab scale vortex milling (Araya et al., 2014) and pilot scale
582 ball milling (Balasundaram et al., 2012), bead milling has been the most studied method. Typically,

583 bead milling uses discs or rings to set in motion the grinding elements inside the milling chamber
584 (Günerken et al., 2015a). Several disruption mechanisms are possible during bead milling. Shear stress
585 due to acceleration of beads towards the mill wall, shear due to centrifugal acceleration of the mill wall,
586 and due to collision of cells with beads due to differences in velocities (Kwade, 1999). The main
587 mechanism of cell disruption is the third, ie cell disruption occurs through beads collisions because of
588 velocity gradients in tangential direction near the stirring disks (Balasundaram et al., 2012; Hennart et
589 al., 2009; Kwade, 1999) leading to combinations of cleavage, fracture and abrasion of cells or particles
590 depending on particle size (Hennart et al., 2009). For large particles of around 15 µm particles
591 disintegration occurs through cleavage and fracture, for medium size particles of around 0.8 µm through
592 cleavage and abrasion, and for small particles only through cleavage (Hennart et al., 2009). Indeed, the
593 disintegration of *Nannochloropsis* sp. in a bead mill, has revealed that larger size cell fractions are
594 disintegrated via impact and compression - correlated to fracture and cleavage- and smaller fractions via
595 shear forces more related to abrasion (Figure 4) (Pan et al., 2017).

596 Table 5

597 Figure 4

598 Since beads transfer energy to the cells their size plays a critical role in process efficiency. Decreasing
599 the bead size from 1mm to 0.3 mm improved the protein release kinetics of *N. oleoabundans* and *C.*
600 *vulgaris* but the effect on the protein yield was less profound (Postma et al., 2017). In the same work
601 the kinetics of carbohydrate release from *C. vulgaris* peaked at a bead size of 0.4 mm and an optimum
602 carbohydrate release yield of approximately 65% was achieved. Bead size did not significantly affect
603 cell disintegration, protein and carbohydrate release of *Tetraselmis suecica* in the same study, indicating
604 a weaker cell wall (Postma et al., 2017). Protein release of 99% for *T. suecica* with a bead size of 0.3
605 mm was achieved at 400 sec of processing time where a plateau is observed. Maximum carbohydrate
606 release did not reach a plateau and was only observed at the end of the experiment, i.e. 900 sec of
607 processing time (Postma et al., 2017). Small bead size also improved the kinetics of *Nanochloropsis*
608 pretreatment in a turbine mill (Pan et al., 2017). More than 90% cell disruption¹ was achieved at 25 min
609 with 0.3-0.4 mm beads, whereas 45 min were required for a similar result with 0.8-1.0 mm size. A
610 further increase of bead size to 1.8-2.0 mm led to a dramatic drop in the mill performance (65% cell
611 disruption at 55 min). Optimal bead sizes for the disruption of *Chlorella* strain P12 with a 4.3 µm cell
612 diameter, depended on the type of instrument used (Doucha and Lívanský, 2008). An optimal bead
613 diameter of 0.3-0.5 mm was found for the Dyno-Mill and LabStar LS 1 bead mills, whereas the optimal
614 bead diameter for the homogenizer MS 18 was 0.5-0.7 mm (Doucha and Lívanský, 2008). However,
615 1.3 mm glass beads were optimum for the bead milling of *P. cruentum* leading to a cell disintegration
616 efficiency of 50% (Montalescot et al., 2015). It is clear from the above that bead size alone cannot
617 efficiently describe the process; other factors, such as the bead density affect the disruption efficiency.
618 Beads with higher densities has been shown to perform better because they can tranfer higher energy to
619 the cells (Hopkins 1991).

620 The milling chamber fill ratio is another important parameter. For *C. vulgaris*, increasing the chamber
621 filling ratio had a positive effect on milling efficiency. For example, when using ZrO₂ beads of 0.3 mm
622 diameter in homogenizer MS 18, increasing the filling ratio from 60% to 75% and 80% increased cell
623 disintegration from 65% to 83% and 85% respectively (Doucha and Lívanský, 2008). Similarly, when
624 using ZrO₂ beads 0.3-0.4 mm in the Dyno-Mill KD 20 S increasing fill ratio from 75% to 85% increased
625 cell disintegration from 75% to 83% (Doucha and Lívanský, 2008). For *P. cruentum* increasing the filling
626 ratio from 35% to 65% not only improved cell disruption, but also led to a shift in the optimum bead
627 size from 1.3 mm to 0.65 mm (Montalescot et al., 2015). Continuous bead milling can be performed in
628 single and multi-pass operation and the flow rate can also affect the performance. Usually increasing
629 the flow rate reduces cell disintegration efficiency, by reducing the residence time in the milling chamber
630 (Doucha and Lívanský, 2008; Montalescot et al., 2015). For example, increasing the feed rate from 12

¹ As determined by microscopic counting, in a hemocytometer, of intact cells before and after disintegration

631 Kg h⁻¹ to 18 Kg h⁻¹ reduced cell disintegration from 92% to 70%. Energetically, it is evident that an
632 optimized process with the shortest duration of treatment is desired and a compromise on the disruption
633 ratio may need to be reached.

634 During bead milling cell disruption² follows first-order kinetics (Doucha and Lívanský, 2008).
635 Disintegration rates of *N. gaditana* show a sharp increase in the initial minutes and a plateau of 95%
636 disintegration is reached at around 20 min (Safi et al., 2017a). Protein release in the same work reached
637 a 50% yield plateau at around 8 min, suggesting that full cell disruption is not always required for
638 efficient extraction of targeted molecules. For *T. suecica* the kinetics of cell wall disruption and protein
639 release are identical and reach a 99% plateau after 6.7 min. A similar carbohydrate release on the other
640 hand is achieved after 15 min without reaching a plateau (Postma et al., 2017).

641 Increasing the solids content of the microalgae biomass slurry has a positive effect both on the yield of
642 extraction and the process energy consumption up to a point. After that threshold the process becomes
643 inefficient. For the extraction of lipids from *Schizochytrium* this limit was 50 g L⁻¹ of biomass (Byreddy
644 et al., 2016), and the disruption of *Nannochloropsis* cells became inefficient after a 18% volume of cell
645 concentration (Pan et al., 2017). Assuming a near water cell density (Mathimani and Mallick, 2018) and
646 a 10% biomass dry weight content, the 18% v/v can be estimated at around 2% DW, a surprising low
647 concentration limit for the process. On the other hand, the extraction of water-soluble protein from
648 *Nannochloropsis gaditana*, was energy efficient at 100 g L⁻¹ but with around 50% protein yield (Safi et
649 al., 2017a).

650 Besides bulk molecules such as lipids and carbohydrates, bead milling pretreatment can improve the
651 yields of high-value molecules found in microalgae. In the lab scale, bead milling of *A. platensis* biomass
652 in 4 cycles of 25 sec at 30 Hz, allowed almost 100% extraction of proteins and phycocyanin at 46 g 100
653 g⁻¹ biomass, and 95 mg g⁻¹ biomass, respectively (Jaeschke et al., 2019). Bead milling pretreatment has
654 also been used in lutein extraction from *Scenedesmus almeriensis*. When the dry biomass was treated
655 for 5min, followed by an alkaline treatment and hexane extraction around 50% of lutein extraction yield
656 was achieved. Addition of alumina powder in the biomass at 1:1 ratio acted as an extra disintegrating
657 agent and increased lutein yield to 98 % (Cerón et al., 2008). Bead milling of *C. vulgaris* for 1 h allowed
658 yields of 50% chlorophyll and 30% carotenoids extracted with supercritical CO₂ and ethanol as a co-
659 solvent (Safi et al., 2014). B-phycoerythrin, a natural high-value pigment was obtained from
660 *Porphyridium cruentum* at a yield of 1.35 mg g⁻¹ of wet biomass after bead milling (Ruiz-Ruiz et al.,
661 2013). Although these results are promising, bead milling pretreatment was used in combination with
662 other treatments, such as freeze drying of the biomass, alkaline pretreatment, and addition of alumina
663 powder as a second disintegration agent. Such steps would increase complexity and cost of an industrial
664 process and their economic and energetic viability would have to be assessed.

665 In the end, bead milling is a very complex process and many more parameters, like bead density,
666 agitation speed, agitators design, and solid content of biomass can affect the outcome. The microalgae
667 cell wall robustness is also a critical factor as it can vary between very weak like *Porphyridium cruentum*
668 to very tough like *Nannochloropsis oculata* (Montalescot et al., 2015). Therefore, optimization can be
669 tedious but also very precise if all parameters are carefully considered. The applicability of statistical
670 design of experiments to such optimisation is evident.

671

672 3.1.2 High-pressure homogenization (HPH)

673 High-pressure homogenization (HPH) is typically used for emulsification purposes but is also suitable
674 for the large-scale disruption of microalgae cells (Günerken et al., 2015a). Typically, a cell suspension
675 is pumped at high pressures through a valve, colliding with an impact ring and then exiting from the
676 high-pressure area into the environment where a high-pressure drop occurs (Middelberg, 1995). Various
677 mechanisms of cell breakage (Figure 5) have been proposed including: shear due to sudden pressure

² As measured by counting undisrupted cells before and after milling in a Bürker chamber

678 drop, shear stress, cavitation and impingement of the cells to the surface of the valve at high velocities
679 (Middelberg, 1995). Several of these mechanisms of cell disruption can occur simultaneously and
680 which one is prevalent may also be species dependent (Middelberg, 1995). However, in most cases cell
681 disruption is caused by shear stress and pressure drop between the valve and the vessel, as well as cell
682 impingement onto the valve walls (Halim et al., 2012). The impingement stress was reported as being
683 proportional to the operating relative pressure in some types of homogenisers (Chisti and Moo-Young,
684 1986). As pressure drop is one of the main cell-breaking mechanisms, the system operating pressure is
685 critical for process performance (Balduyck et al., 2018; Halim et al., 2012; Onumaegbu et al., 2018;
686 Samarasinghe et al., 2012). Increasing the pressure in the HPH of *Chlorella sp.* from 250 to 1400 bar
687 increased cell rupture³ from 10% to 80% (measured by counting undisturbed cells before and after HPH
688 in a Neubauer haemocytometer chamber) and subsequently the recovered lipids from 20% to 100% (Yap
689 et al., 2014). Similarly, in the HPH treatment of *Desmodesmus sp.* F51 increasing the pressure from
690 689.5 to 2758 bar increases the release of carotenoids from around 0.02 mg g⁻¹ to around 0.28 mg g⁻¹
691 and the release of chlorophylls from around 0.01 mg g⁻¹ to almost 1.5 mg g⁻¹ (Xie et al., 2016). Complete
692 disruption of *N. gaditana* cells occurred at 1500 bar. However, the protein release had already peaked
693 at a much lower pressure (1000 bar), with a yield of 50%, indicating that partial cell breakage is enough
694 for the sufficient extraction of desired compounds (Safi et al., 2017a). As with other disruption and
695 extraction methods, the method's influence on the target compound degradation is an important quality
696 factor to consider during a biorefinery process.

697 Figure 5

698 The number of passes also has a positive impact on HPH extraction yields (Balduyck et al., 2018;
699 Samarasinghe et al., 2012). Increasing the number of passes from 1 to 4 increased carotenoid release
700 from *Desmodesmus sp.* F51 from around 0.28 mg g⁻¹ to around 0.42 mg g⁻¹, and chlorophyll release
701 from 1.5 mg g⁻¹ to 2.4 mg g⁻¹ (Xie et al., 2016). Five passes were optimal for release of carbohydrates
702 and proteins from dilute solutions of *C. vulgaris* treated at 1500 bar (Carullo et al., 2018). After five
703 passes of *Chlorococcum sp.* cells more than 90% disruption was achieved (Halim et al., 2012). Increasing
704 the number of passes to 6 had a positive effect on both the total protein and reducing sugars extraction
705 yields from HPH treated *N. oculata* (Shene et al., 2016). Pretreatment of *Nannochloropsis sp.* with HPH
706 at 150 MPa required 6 passes to maximize extraction of green and red pigments, as deduced from
707 increases in spectral intensities of the extracts at 415 nm and 620 nm, respectively (Grimi et al., 2014).
708 In general, HPH exhibits a first order decrease of cell disruption rate after each passage through the
709 homogenizer (Halim et al., 2012). The rupture of *Nannochloropsis sp.*, *Chlorella sp.* and *T. suecica* cells
710 followed an exponential decay as a function of the number of passes (Spiden et al., 2013).

711 Smaller effects on HPH performance were attributed to cell concentration and culture stress level
712 (Samarasinghe et al., 2012). Increasing the cell concentration of *Desmodesmus sp.* F51 from 2 and up
713 to 90 g/L did not affect the particle size distribution of HPH treated cells nor the release of carotenoids
714 and chlorophylls (Xie et al., 2016). Increasing the cell concentration of *N. oculata* from 1.78% up to 8%
715 w/w had little effect on the yield of lipids extracted (Shene et al., 2016). Indeed, it was found for
716 *Nannochloropsis sp.* that important processing parameters such as flow rate, power draw and disruption
717 efficiency were independent of cell concentration up to 25% w/w (Yap et al., 2015). As 25% is a typical
718 concentration target of many harvesting/dewatering methods the ability to effectively break cells at these
719 concentrations makes HPH a potential method for industrial microalgal cell disruption.

720 The method is also affected by the microalgae species biological properties. More specifically by cell
721 wall toughness and its resistance to disruption. For example, only 170 bars are enough to break *T.*
722 *suecica* cells, whereas *Chlorella sp.* cells require 1070 bar, and *Nannochloropsis sp.* 2000 bar (Spiden
723 et al., 2013).

724

³ As measured by counting undisturbed cells before and after HPH in a Neubauer haemocytometer chamber

725 3.1.3 Ultrasonication

726 Two major mechanisms of cell disruption are reported during ultrasonication. Generation of high-
727 pressure bubbles and their cavitation, which generates shock waves that cause high shear forces (Gerde
728 et al., 2012; Günerken et al., 2015b; Soo Youn Lee et al., 2017). The additional effect of free radical
729 generation and oxidative degradation of cells has also been reported (Gerde et al., 2012). Increasing
730 ultrasonication power and treatment time have a positive effect on cell disruption (Greenly and Tester,
731 2015; Keris-Sen et al., 2014; Meng Wang and Yuan, 2015; B. Zhang et al., 2018). Increasing the power
732 increases bubble generation but reduces pressure inside the bubbles (Wang and Yuan, 2016). Beyond
733 the optimum power and time values, undesirable effects on released products can occur, such as
734 oxidative degradation (Gerde et al., 2012), and reduction of fatty acids chain size and double bond
735 saturation (Cheng et al., 2014). Increasing the frequency of ultrasonication, positively affects cell
736 disruption. Optimum sonication frequency is species depended and should be determined for each
737 microalgae species (Kurokawa et al., 2016). Application of a pulsed mode improved protein extraction
738 yield from *C. vulgaris* FSP-E (Chia et al., 2019). The pulsed mode has many advantages such as energy
739 input reduction, lower heat generation during treatment, hence reduction in the extracted protein
740 denaturation. This is important as temperature during ultrasonication can reach as high as 93 °C if not
741 controlled (de Farias Silva et al., 2020). Ultrasonication efficiency seems to be independent of cell
742 concentration at low to medium concentrations (1.5 to 14.1 g L⁻¹) (Gerde et al., 2012). At higher cell
743 concentrations a significant reduction in disruption efficiency can occur (Greenly and Tester, 2015;
744 Meng Wang and Yuan, 2015) and has been attributed either to increased viscosity or reduced energy
745 input per cell (Meng Wang and Yuan, 2015). Cell disruption in ultrasonication follows first order
746 kinetics. The constant of disruption rate is directly proportional to the power level and has a parabolic
747 relationship to cell concentration (Halim et al., 2013).

748 Figure 6

749 Cell aggregate formation during flocculation adversely affects cell disruption by ultrasonication. Cell
750 aggregation reduces the penetration of ultrasonic power and protects cells inside the aggregate from
751 bubble generation and collapse (Wang et al., 2015). The formation of microalgae cell aggregates by
752 flocculation can occur naturally or be induced during harvesting/concentration (Muylaert et al., 2017).
753 As flocculation is one of the most efficient concentration methods of microalgae biomass, its negative
754 effect on cell disruption by ultrasonication demonstrates that careful selection of all downstream
755 processes is required when assembling multiple methods in the same process.

756 Ultrasonication performed poorly in many comparative studies of cell disruption methods. It was less
757 effective compared to microwave, electroflotation and thermal treatment (Florentino de Souza Silva et
758 al., 2014), microwave and thermal treatment (McMillan et al., 2013), High-Pressure Homogenization,
759 sulfuric acid and bead beating (Halim et al., 2012), Pulsed Electric Field, and High-Pressure
760 Homogenization (Grimi et al., 2014). For example, ultrasound was not effective when applied in the
761 pretreatment of microalgae with a very resistant cell wall such as *H. pulvialis* cysts. Even at 600 W for
762 30 min at 20 kHz frequency and pulses 1 sec on 3 sec off a very low 12% astaxanthin yield was observed
763 (Liu et al., 2018). In another study 80% extraction efficiency of astaxanthin was achieved only when
764 ultrasound pretreatment of the *H. pluvialis* biomass was performed in a 2M sodium hydroxide solution
765 (Haque et al., 2016). Although careful optimization of all these methods is required before safe
766 conclusions can be drawn, it seems that ultrasonication is not a very effective method for cell disruption.
767 It also suffers from the inability to handle large cell concentrations. Despite that, it can be useful in
768 combination with other methods as it can enhance the disruption efficiency of other pretreatment
769 methods. This will be discussed in following sections.

770

771 3.1.4 Pulsed Electric Field

772 Pulsed Electric Field is based on the exposure of microalgae cells to an intense electric field for very
773 short durations (pulses). When the applied electric field is above a threshold it can induce reversible or
774 irreversible pores creation (electroporation) on the cell membranes. Irreversible electroporation can

775 facilitate intracellular material extraction (Figure 7) (Luengo et al., 2015). The microalgae suspension
776 must not contain ions in order to be non-conductive. The medium of marine microalgae that contains
777 significant salt concentrations, must be removed before PEF treatment, for example by electro dialysis,
778 or cell washing and resuspension, increasing process steps, cost and energy. PEF is therefore more
779 suitable for freshwater microalgae treatment (Günerken et al., 2015b). The cell membranes
780 permeabilization (electroporation) is dependent on the electric field energy (Carullo et al., 2018). The
781 main effect of PEF is pore creation in the cell membranes and does not lead to total cell disruption and
782 cellular debris generation (Carullo et al., 2018). Temperature increase during PEF treatment is generally
783 small (Picart and Cheftel, 2003), for example during PEF treatment of *C. vulgaris* the sample
784 temperature increased from 20 °C to 38 °C depending on the operating parameters; the effect of this
785 temperature increase on the yield of extracted proteins and their functionality were not investigated
786 (Scherer et al., 2019). When very high treatment intensities were applied, e.g. higher than 35 kWh m⁻³
787 in the PEF treatment of *Synechocystis* PCC 6803, it led to limited cell disruption (approx.10%), while
788 the cell majority (around 87%) remained intact but with damaged membranes (Sheng et al., 2011).

789 Figure 7

790 PEF efficiency is affected by the microalgae culture's growth state and cell size. Larger cells are more
791 susceptible to electroporation, whereas smaller cells are more resistant (Safi et al., 2017a). The large size
792 of *H. pulvialis* cells (20 µm) may, therefore, have aided PEF treatment, as a 96% astaxanthin extraction
793 efficiency, using ethanol, was reported (Martínez et al., 2019). In contrast, the small cell size (2-8 µm)
794 of *C. sorokiniana* (Azaman et al., 2017) may have a negative impact on PEF treatment. For example, a
795 yield of 3.25 mg g⁻¹ DW of pigments was achieved after PEF treatment, an improvement of 14%
796 compared to the untreated sample (Leonhardt et al., 2020). Both species have rigid cell walls; *H.*
797 *pulvialis* with a resistant algaenan layer, and *C. sorokiniana* a glucosamine rich and chitin-like layer.
798 Cell wall rigidity can lead to inefficient extraction of molecules even at very high intensities and number
799 of pulses (Safi et al., 2017a). This has been confirmed by comparing the PEF efficiency between a wild
800 type strain of *C. reinhardtii* with cell wall and a mutant without cell wall (T Lam et al., 2017). A dramatic
801 increase on protein extraction yields was observed in the cell wall free mutant even at very low power
802 intensities. The effect could be repeated when the wild strain's cell wall was removed by enzymatic
803 digestion.

804 Due to its relatively mild effect on cell integrity, PEF is often described as a mild or selective
805 pretreatment method that can specifically target intracellular molecules, such as low molecular weight
806 proteins and carbohydrates (Carullo et al., 2018; Safi et al., 2017a; T Lam et al., 2017). Although a
807 significant increase in the release of water-soluble intracellular components is induced by PEF, their
808 yields are relatively low (around or below 10%) even at relatively high intensities indicating only partial
809 success of PEF as a pretreatment method. In all these studies the treated microalgae had a full cell wall,
810 that may explain the low yields obtained.

811 The low levels of lipids released with PEF from *Auxenochlorella protothecoides* cells is possibly
812 because the generated pores are more permeable to smaller water-soluble molecules rather than the
813 larger lipid droplets (Eing et al., 2013). This can enable an easier separation of carbohydrates and
814 proteins from lipids that could be sequentially extracted by organic solvents (Eing et al., 2013). PEF
815 treatment of wet *Synechocystis* PCC 6803 biomass increased the lipid yields and lowered the solvent to
816 biomass ratio from 10 to 5 as it allowed the penetration of isopropanol through the pores to extract lipid
817 molecules (Sheng et al., 2011). The kinetics of lipid extraction from *Ankistrodesmus falcatus* with the
818 green solvent ethyl acetate were relatively slow and around 2h were required for the process to plateau
819 at a yield of around 2500 µg L⁻¹. After PEF treatment the lipid extraction was almost time independent
820 as increasing extraction time between 0 and 2 h lead to a marginal increase in lipids from 9000 µg L⁻¹
821 to almost 9800 µg L⁻¹ (Zbinden et al., 2013). Although a yield increase is also shown, extraction
822 experiments with and without PEF treatments were performed with different microalgae samples, and
823 therefore the effect of PEF on the yield remains inconclusive (Zbinden et al., 2013). Similarly, the PEF
824 treatment of *A. protothecoides* increased lipid extraction with 100% ethanol from around 30 to around
825 220 mg g⁻¹ of dry weight biomass (Eing et al., 2013).

826

827 3.1.5 Drying

828 Drying is the decrease of microalgae biomass moisture content to around 10%. It is a post harvesting
829 and dewatering process aims at improving the stability of the final product. The main challenges of
830 drying are the long time and high energy input required and the associated cost increase. The simplest
831 drying technology is sun drying as it requires low capital and operating cost (Ansari et al., 2018).
832 However, long process time is needed for efficient water removal from the sample, even as high as 72
833 h (Guldhe et al., 2014). Sun drying can decrease the extraction yield of biomolecules from microalgae
834 compared to untreated biomass (Ansari et al., 2018) possibly due to degradation reactions or due to
835 consumption from microbial contamination during the long drying times. Convection drying utilizes hot
836 air to remove moisture from the biomass. It has shorter process times, up to 12h and leads to less
837 biomolecule degradation compared to sun drying (Ansari et al., 2018). The lipids extracted from oven
838 dried biomass has slightly lower saponification and acid values compared to sun dried biomass,
839 indicating lower free fatty acid values.

840 Freeze drying, also called lyophilization, is a very mild drying method that does not destroy the
841 biomolecules of the biomass samples (Ansari et al., 2018). It is based on the sublimation of ice from a
842 frozen sample into the vapor phase inside a vacuum. Freeze-drying can have long process time, usually
843 around 24h. Due to the requirement for deep freezing of the sample, and maintaining a vacuum and a
844 negative temperature in the freeze-drier, it is the most energy consuming and expensive method,
845 typically 1 or 2 orders higher than the rest (Table 6). Its long process time, high energy requirement and
846 high cost should limit application of freeze-drying for very sensitive and high-value products. Spray
847 drying is based on heating of the microalgae slurry as it passes through a spraying nozzle which leads
848 to the formation of a dry powder as the moisture rapidly evaporates from the sample droplets. Samples
849 of freeze-dried *P. tricornutum* cells were found more prone to lipolysis, and spray dry lipolysis ed
850 samples were more sensitive to oxidation caused by the reduction of the antioxidant carotenoid content
851 (Ryckebosch et al., 2011). Drum drying is a mature drying technology with applications in the food
852 industry. It has lower energy requirements and costs compared to spray drying (Table 6) but can process
853 only up to 1000 Kg h⁻¹ of sample compared to the 10000 Kg h⁻¹ maximum upper limit for spray drying.
854 This big difference in processing capacity if favor of spray drying can compensate for the slightly higher
855 energy and operational costs, and make spray drying an attractive drying method (Fasaei et al., 2018).

856 Drying microalgae before extraction of valuable components clearly adds to the treatment cost while
857 greatly increasing the extraction efficiency. Recent technology on extraction from wet biomass is
858 promising but the cross-over point where the lower extraction efficiency of wet extraction can be
859 accepted has not been reached.

860

861 Table 6

862

863 3.2 Chemical methods

864 3.2.1 Hydrothermal

865 Cell wall disruption via hydrolysis is variably referred in the literature as water bath treatment (McMillan
866 et al., 2013), autoclave (Florentino de Souza Silva et al., 2014), hydrothermal pretreatment (Xiao et al.,
867 2019) and steam explosion (Lorente et al., 2018). In all these variations two main mechanisms are
868 thought responsible for the microalgae cell disruption; cell wall rupture due to internal pressure build-
869 up from the heating, and hydrolysis of cell wall components. Due to this dual effect the method can be
870 classified both as physical and chemical. At lower temperatures where hydrolytic reactions of the cell
871 wall components are not yet favoured disruption due to internal pressure build-up is the prevailing
872 mechanism (McMillan et al., 2013). Even at such mild treatment conditions, e.g. 90 °C, for 20 min,

873 extensive disruption⁴ of 87% for *N. oculata* cells was observed. At higher temperatures the hydrolysis
874 reactions of the microalgae structural components such as the cell wall polysaccharides becomes
875 significant, leading to the production of monomeric sugars and the reduction of cell wall integrity (Xiao
876 et al., 2019). In the steam explosion variation of the method the microalgae slurry is heated under
877 pressure with saturated steam to the desired temperature, and at the end of the reaction the slurry
878 undergoes rapid decompression (explosion) through a release valve (Lorente et al., 2017). The rapid
879 change in pressure can create an additional cell disruption effect.

880 The hydrothermal method has been extensively used to pretreat lignocellulosic biomass, in order to
881 remove hemicellulose (Garrote et al., 1999) and enhance the enzymatic digestibility of cellulose (Nitsos
882 et al., 2013), and to subsequently increase the bioethanol (Ruiz et al., 2012) and biomethane (Antwi et
883 al., 2019) production yields. The method can also be applied to microalgae that have a carbohydrate cell
884 wall composition. For example, it is very efficient in the release of carbohydrates from microalgae.
885 Carbohydrate yields of 44 % of total carbohydrate content from *N. gaditana* (Lorente et al., 2015), 60%
886 (Fu et al., 2018) and 80 % (Xiao et al., 2019) from *C. pyrenoidosa*, 97 % from *C. vulgaris* (Xiaojian et
887 al., 2017) have been achieved. At prolonged pretreatment times the released biomolecules can further
888 react to form degradation products (Córdova et al., 2018), such as furans and organic acids from the
889 dehydration (Horvat et al., 1985; Mussatto and Roberto, 2004; Palmqvist and Hahn-Hägerdal, 2000), or
890 from Maillard reactions (Fu et al., 2018) of the carbohydrates with amine containing groups. Bioethanol
891 production from hydrothermally treated *Schizocytrium* sp. slurry after enzymatic hydrolysis reached
892 90% of the theoretical maximum ethanol yield (Kim et al., 2012). Hydrothermal pretreatment increased
893 the yield of biogas from the anaerobic digestion of microalgae biomass by 11% (Martín Juárez et al.,
894 2018) and 28% (Passos et al., 2015) for mixed microalgae biomass and 57% for pure *C. pyrenoidosa*
895 (Xiao et al., 2019). The production of toxic compounds due to the released molecules degradation during
896 steam explosion led to a reduction of biomethane yield due to anaerobic digestion inhibition (Córdova
897 et al., 2018).

898

899 Table 7

900

901 Two stages can be observed during hydrothermal treatment of microalgae based on the temperature
902 regimes. Initially and up to 100 °C the release of high molecular weight intracellular components such
903 as starch and protein occurs; the gelatinization of starch and denaturation of proteins can lead to an
904 increase in the medium viscosity; at 140-160 °C the macromolecules are hydrolyzed into oligomers of
905 lower molecular weight and the viscosity decreases (Chen et al., 2019a; McMillan et al., 2013). The
906 microalgae slurries produced from hydrothermal pretreatment, exhibited shear thinning behavior,
907 suggesting non-Newtonian fluid behaviour (Chen et al., 2018). The ability of viscosity reduction by
908 increasing the temperature or the shear rate can be valuable as it would allow the processing of high
909 biomass concentrations in downstream unit operations.

910 A kinetic study showed that the treatment temperature, time and pressure were the most important
911 parameters of hydrothermal pretreatment in terms of carbohydrate release, whereas temperature was the
912 main factor that controlled protein release (Xiaojian et al., 2017). This dependence of carbohydrate
913 release on time and temperature is similar in the hydrothermal pretreatment of lignocellulosic biomass,
914 due to the hydrolytic reaction that dominates it. To express the combined effect of time and temperature
915 the severity factor ($\log R_0$) has been established in the hydrolysis of lignocellulosic materials, that can
916 be calculated from the equation

⁴ As measured by counting the number of intact cells treated after a specific time interval against those initially determined from the control, using a microscope

917
$$R_o = t * \exp\left(\frac{T_{(t)} - 100}{14.75}\right)$$

918 where, t is the pretreatment time and $T_{(t)}$ the pretreatment temperature for holding time t.

919 It is possible that due to similarities in the chemical composition of carbohydrates in plants and
 920 microalgal cell walls, the equation can correlate the effects of microalgae pretreatment with time and
 921 temperature. Some evidence towards that can be seen in Table 7. Although the data points correspond
 922 to yields from different studies, with different microalgae species, e.g. *C. vulgaris* (Mendez et al., 2013),
 923 *N. gaditana* (Lorente et al., 2015) *C. pyrenoidosa* (Xiao et al., 2019), different mixing and heating
 924 régimes, e.g. stirring in reactor (Qu et al., 2018), lack of mechanical mixing in steam explosion (Lorente
 925 et al., 2015) and biomass concentrations ranging from 16 g L⁻¹ (Mendez et al., 2013) up to 20% w/w
 926 (Qu et al., 2018)), the increase of biomolecules release or extraction yield with increasing severity can
 927 be clearly seen.

928 Significant protein release from hydrothermally treated microalgae biomass is also possible; 47 % (Xiao
 929 et al., 2019) and 83% (Fu et al., 2018) of protein was isolated from *C. pyrenoidosa*, and 76% from *C.*
 930 *vulgaris* (Xiaojian et al., 2017). Lower protein recovery (approx. 20%) is observed at lower severity
 931 factor (logRo) values (approx. 2). As with carbohydrates, protein release is also well correlated to the
 932 treatment's severity factor. Cell wall disruption also allowed the subsequent extraction of lipids, with
 933 yields of 60% from *N. salina* (Lee and Han, 2015a), 68% from *C. vulgaris* (Xiaojian et al., 2017) and
 934 84% from *Nannochloropsis* sp. (Qu et al., 2018) reported. After the hydrothermal pretreatment of *C.*
 935 *vulgaris* it was shown that the lipids remained in the biomass and were not detected in the pretreatment
 936 liquid that was rich in carbohydrates and protein (Xiaojian et al., 2017). This may allow the selective
 937 separation of water-soluble molecules and lipids, in comparison to other cell disruption methods like
 938 bead beating that generate difficult to separate water-oil emulsions.

939 Other applications include the extraction of carotenoids from *H. pluvialis* at yields higher than 85%
 940 (Mendes-Pinto et al., 2001). Near complete extraction of astaxanthin was shown by hydrothermal
 941 disruption of *H. pluvialis* cells at 200 °C for 10 min (logRo 4) (Cheng et al., 2017). The chemicals
 942 released from hydrothermal treatment of *H. pluvialis* at 200 °C for 20 min (logRo 4.2) showed significant
 943 antioxidant and antimicrobial activity. The antimicrobial activity was attributed to extracted short chain
 944 fatty acids, while the antioxidant activity was to vitamin E, phenolics, as well as carbohydrate degradation
 945 products (Rodríguez-Meizoso et al., 2010). The extraction of phenolic compounds from *Chlorella* sp.
 946 by mild hydrothermal treatment is also possible. Treatment of *Chlorella* sp. biomass at a concentration
 947 of 20 wt. % for 5 min at 163 °C (logRo 2.6), yielded 58.73 mg of gallic acid equivalent g⁻¹ that exhibited
 948 significant antioxidant activity (inhibiting by 68.5% the DPPH radical) (Zakaria et al., 2017).
 949 Temperature had a significant effect on extraction yield. Increasing the temperature from 100 °C to 170
 950 °C increased both the total phenolic content and the antioxidant activity of the extracts. Further increases
 951 of the temperature up to 250 °C led to a decrease of the phenolic content and antioxidant activity by
 952 almost 50%, to levels comparable to those at 100 °C. (Zakaria et al., 2017). This was attributed to thermal
 953 degradation of the extracted phenolics. Increase of time from 5 to 20 min decreased phenolic content
 954 and antioxidant activity of the extracts by approximately 10% (Zakaria et al., 2017). Increasing the
 955 biomass concentration from 5 wt. % to 20 wt. % increased phenolic content by approx. 50%. Antioxidant
 956 activity, however, peaked at 12.5 wt. % and remained almost constant up to 20 wt. % of biomass
 957 concentration, but no explanation for this phenomenon was offered (Zakaria et al., 2017).

958 Hydrothermal treatment of biomass at very high temperatures (above 230 °C -supercritical water) can
 959 lead to cellular structure disintegration through biomass carbonization, that can also be beneficial for
 960 lipid extraction (Kröger et al., 2018). At even higher temperatures (e.g. 260, 300, 340 °C) the biomass
 961 undergoes hydrothermal liquefaction (HTL) and is directly converted to hydrothermal bio-oil that can
 962 be used as a biofuel (B. Zhang et al., 2018). This is no longer a pretreatment method but a direct
 963 conversion method of microalgae biomass into the final product. Its main advantages are that water
 964 removal is not required and that it is a single step process, but it is limited to biofuel production, and
 965 therefore maybe more suited for lower value microalgae or microalgae debris after the extraction of
 966 valuable molecules.

967 Overall hydrothermal pretreatment has many advantages including lack of reagents other than water,
968 ability to operate at high solids content, ability to pretreat a variety of microalgae species and ease of
969 post treatment separation and fractionation of compounds. Careful optimization is required, to minimize
970 degradation of valuable products due to the high temperatures employed. Another disadvantage is the
971 energy input required for the reaction to take place, however, significant energy can be recovered by
972 heat exchange (Chen et al., 2019b) and even solar heated hydrothermal reactors showed significant
973 extraction yields minimizing energy requirements of the process (Xiao et al., 2019).

974

975 3.2.2 Acid treatment

976 The pretreatment of microalgae with dilute solutions of inorganic acids can be considered as a
977 hydrothermal pretreatment variation. The rate of hydrolytic reactions at elevated temperatures in water,
978 are enhanced by the acid catalyst's addition. High carbohydrate, lipid and protein yields can be achieved
979 at lower temperatures and shorter reaction times. Acid concentration employed is usually between 1-5
980 % and temperatures between 110°C and 150°C have been reported; but the specific conditions must be
981 tailored to the target molecules to be extracted. Sulfuric acid is most commonly used for acid
982 pretreatment due to its low cost and efficiency. Other acids such as nitric acid have also been tested, but
983 significant reduction in the yield has been observed at higher acid concentrations (Lee et al., 2014; Lee
984 and Han, 2015b). This is probably a result of the nitric acid's oxidative action that would degrade the
985 released molecules. Hydrochloric acid gave superior saccharification results compared to sulfuric acid
986 and nitric acid at concentrations ranging from 0.2 M to 2M, in the hydrolysis of *Hindakia tetrachotoma*
987 *ME03* biomass (Onay, 2019).

988 A modified version of the severity factor ($\log R_o$) from the hydrothermal pretreatment can be expressed
989 in terms of time (t), temperature (T) and acid catalyst concentration (related to pH). The Combined
990 Severity Factor (CSF) is given by the equation:

$$991 \quad CSF = \log \left[t \exp \left(\frac{T - 100}{14.75} \right) \right] - pH$$

992

993 A good correlation between the process CSF and the carbohydrate release or the lipid extraction yields
994 can be obtained (Table 8). For *N. gaditana* a carbohydrate yield of 50 % was achieved at a CSF 0.4 and
995 increased to 59%, 63% and 89.7% for CSFs of 0.75, 1.0 and 1.4, respectively (Lorente et al., 2015). The
996 pretreatment of *Chlorella sorokiniana* and *P. tricornutum* at CSF of 1.4, leads to almost complete
997 carbohydrate recovery yields (95% and 96%, respectively). The big advantage of the process is that
998 these high carbohydrate extraction yields are achieved only after 5 min of pretreatment time. At higher
999 CSFs of 1.87 both *N. gaditana* and *C. sorokiniana* showed reduced carbohydrate yields, at 69% (Lorente
1000 et al., 2017) and 79.2 % (Lorente et al., 2018) respectively; possibly due to released sugar degradation.
1001 Released carbohydrates from microalgae have been used to produce bioethanol with increased yield
1002 compared to the untreated biomass (Choi et al., 2010; Ngamsirisomsakul et al., 2019). The degradation
1003 products generated from acid hydrolysis can lead to inhibition of fermentation-based microalgae
1004 valorisation processes (Martín Juárez et al., 2018). Pretreatment liquid neutralization may also be
1005 required as the low pH values can significantly reduce microbial growth.

1006

1007 Table 8

1008 Lipid extraction yields remain relatively low (< 30%) below CSF values of 1.4, whereas lipid yields up
1009 to 88% are reported at CSF of 1.9 (Lee et al., 2014). This indicates that relatively high cellular wall
1010 degradation is required to enable efficient intracellular lipids solvent extraction Although the degree of
1011 cell disruption is not reported by the authors, it is possible that at these higher CSF' values where lipid
1012 extraction is efficient, extensive cell wall disruption has occurred. Optimal carbohydrate-hydrolysis and
1013 lipid-extraction yields seemed to occur in a very narrow parameter range (CSF values between 1.4 and

1014 2). This can lead to severe degradation of the released products even with minute changes in the process
1015 control. Moderate protein release around 50% is also possible with CSF values of approximately 2
1016 (Lorente et al., 2017).

1017 Acid treatment has also showed promising results in extraction of high-value molecules. Astaxanthin
1018 extraction from *H. pluvialis* by treatment with 4M HCl at 70 °C for 60 min (CSF 1.5) achieved a yield
1019 of 80% (Liu et al., 2018). Lutein extraction yield of 3.5 mg g⁻¹ *C. pyrenoidosa* biomass was achieved
1020 with 16% HCl treatment for 41 min (Arun, 2017).

1021 In general, dilute acid pretreatment is a very fast and efficient pretreatment method for the extraction of
1022 carbohydrates and lipids from microalgae. Application for extraction of high value molecules may also
1023 become possible in the future. However, due to the acids aggressive nature, careful pretreatment
1024 conditions optimization is required to avoid the extracted molecules degradation, especially the proteins,
1025 antioxidants, and pigments.

1026 3.2.3 Alkaline treatment

1027 Alkaline treatment involves biomass exposure to a basic aqueous medium at elevated temperature. Like
1028 acid hydrolysis, it can also be considered as a hydrothermal pretreatment variation with addition of a
1029 basic catalyst. A fundamental difference concerns the operatory condition of these pre-treatments,
1030 especially adapted for lower temperatures, (below 120 °C), and longer treatment time that can span up
1031 to several days (Mahdy et al., 2014a). Alkaline pretreatment can target the hydrolysis of ester bonds and
1032 hydrogen bonds between polysaccharide and non-polysaccharide cell wall components, especially at
1033 lower temperatures (Costa and Plazanet, 2016). For this reason, it has been extensively used for the
1034 extraction of hemicellulose from lignocellulosic biomass. At low temperature microalgae alkaline
1035 pretreatment induces a mercerization-like effect accompanied by cell wall swelling and an improvement
1036 of protein extraction yields (Phong et al., 2018). At higher temperatures intramolecular glycoside bonds
1037 are also affected leading to the hydrolysis of cell wall and intracellular carbohydrates.

1038 Like acid pretreatment the combined effect of time (t), temperature (T) and base catalyst concentration
1039 (pOH), can be expressed by a modified Combined Severity Factor (CSF') that is given by the equation:

1040

1041

$$CSF' = \log \left[t \exp \left(\frac{T - 100}{14.75} \right) \right] - pOH$$

1042

1043 The CSF' can describe the effect of pretreatment on the hydrolysis of carbohydrates, release of protein
1044 and lipid extraction from microalgae. Again, a narrow range of CSF' values (between 1.2 and 2.5)
1045 provides the optimal carbohydrate hydrolysis yields (80 to 100 %) that have been reported.
1046 Approximately 80% of total carbohydrates were recovered in the hydrolysates of *H. tetrachotoma* ME03
1047 (Onay, 2019) and a mixed microalgae culture (Shokrkar et al., 2017) at CSF' 2.4 and 2.5, respectively.
1048 Complete carbohydrate recovery was possible for *C. vulgaris* ESP6 at a much lower CSF' of 1.3 (Liu et
1049 al., 2012). Protein release yields from a mixed extraction yield was moderate at 32%, however very low
1050 CSF' values were tested (CSF' < -2.9). These values were obtained by pretreatment temperatures up to 75
1051 °C and prolonged pretreatment times of up to 48h; CaO (lime) was used as the alkaline catalyst. Similar
1052 results were found for the pretreatment of *C. vulgaris* with 42% protein yield at CSF' -1.8 (Mendez et
1053 al., 2013). The extraction of lipids by enzymatic hydrolysis of alkaline pretreated *Nannochloropsis* sp.
1054 was comprehensively studied for a variety of temperature, time and pH pretreatment conditions (Wu et
1055 al., 2017). Alkaline treatment was followed by an enzymatic hydrolysis step with a cocktail of
1056 commercial enzymes (cellulase, protease, lysozyme, and pectinase). The results show a very good
1057 correlation with CSF'. Maximum lipid extraction yields were obtained at low CSF' values, between -0.9
1058 and -0.3, much lower compared to those required for maximum carbohydrate release shown above. They
1059 are also lower compared to the CSF values required for quantitative lipid extraction by acid pretreatment.
1060 This could be due to differences in cell wall resilience between species. Also, the results of this study

1061 are not directly comparable to the rest alkaline pretreatment studies as it employs an extra enzymatic
1062 hydrolysis process step, after alkaline treatment, leading to increased cell wall degradation and easier
1063 lipid extraction. Further increasing the severity beyond -0.3 leads to a reduction in the lipid yield,
1064 probably due to lipid degradation.

1065 Alkaline pretreatment is also used to enhance the production of bio-methane from microalgae anaerobic
1066 digestion. The pretreatment of *C. vulgaris* and *Scenedesmus* sp. at low CSF' values led to marginal
1067 increases in the methane yield (20% improvement at the base case) (Mahdy et al., 2014a). Similar results
1068 were obtained in the alkaline pretreatment of mixed microalgae culture at low CSF' (0.24) that led to a
1069 25% improvement of methane yield (Solé-Bundó et al., 2017). The NaOH pretreatment of *C. vulgaris*
1070 at CSF' -1.8 gave a 68.8% methane yield (Mendez et al., 2013). NaOH pretreatment of mixed microalgae
1071 biomass significantly increased methane production from approximately 162 mL CH₄ g⁻¹ of volatile
1072 solids (VS) to 377 mL CH₄ g⁻¹ VS, leading to 91% of biodegradability (Martín Juárez et al., 2018). The
1073 enhanced extraction of the carotenoid lutein from *Scenedesmus almeriensis* by alkaline pretreatment has
1074 also been studied (Cerón et al., 2008). The recovery yield can be related to CSF' over a very narrow
1075 range, between -2 and 0 with the maximum recovery of 90% achieved at -0.8 The rapid lutein yield
1076 decrease shows that optimization of parameters is critical for extraction of sensitive molecules to avoid
1077 loss through degradation reactions.

1078

1079 3.2.4 Oxidative pretreatment

1080 3.2.4.1 Ozonolysis.

1081 Exposure to oxidative agents can be a very aggressive treatment of biological materials. It has been
1082 traditionally used in the pulp and paper industry for pulp bleaching, as a more environmentally friendly
1083 alternative to traditional chlorine-based processes (Rounsaville and Rice, 1996). Bleaching is achieved
1084 by removing the residual and recalcitrant lignin that remains after the pulping process. It has also been
1085 applied as a pretreatment method of lignocellulosic biomass to enhance enzymatic hydrolysis (García-
1086 Cubero et al., 2009), and as a cell disruption method for treatment of microalgae (Keris-Sen and Gurol,
1087 2017). As with pretreatment of lignocellulosic biomass, ozonolysis of microalgae cells can increase the
1088 enzymatic cell wall saccharification leading to 81% glucose yields. Pretreatment efficiency increases
1089 with the ozone dose administered up to a maximum and then gradually decreases. Low glucose yields
1090 at low ozone doses is attributed to insufficient cell wall disruption, whereas low yields at high ozone
1091 dosages is the result of carbohydrates degradation (Keris-Sen and Gurol, 2017). Thus, an ozone dose
1092 optimization is to be determined in order to improve the yield of glucose.

1093 Exposure of microalgae cells to ozone could be performed by conventional bubbling of ozone or under
1094 pressure assisted ozonation with compression and decompression cycles (Huang et al., 2014).
1095 Ozonolysis leads to cell rupture and the release of intracellular material in the medium that is
1096 accompanied by measurable increases of total organic compounds, nitrogen and phosphorus. Increasing
1097 the ozone pressure as well as the number of compression and decompression cycles is beneficial for the
1098 lipid extraction; optimization of the two parameters led to 27% lipid yield (g g⁻¹ biomass) (Huang et al.,
1099 2014). Two mechanisms of pretreatment were identified. Under conventional ozonation, oxidation
1100 increases cell wall permeability and cytoplasm release. In the pressure assisted system, compression
1101 increases dissolved gas inside the cytoplasm and during decompression gas expansion occurs. Repetition
1102 of the compression decompression cycle induces additional stress to the cells and increases disruption
1103 efficiency and lipid extraction yields (Huang et al., 2014).

1104 Ozonation of the filamentous cyanobacteria *A. platensis* has been investigated in various ozone doses.
1105 At low ozone concentrations (5-15 mg L⁻¹) the dominant effect observed was the filament's shear into
1106 smaller fragments. At higher ozone doses (up to 510 mg L⁻¹) an increase in filament transparency
1107 accompanied by the release of intracellular organic material was observed; these cellular material
1108 subsequently formed clusters (Akao et al., 2019). Similar effects were observed in the ozonation of
1109 mixed microalgae cultures for the production of methane through anaerobic digestion (Cardeña et al.,

1110 2017). At optimal ozonation pretreatment conditions a 66% increase in the methane yield was obtained.
1111 Under these conditions cells exhibited fragmentation in the cell walls due to oxidation leading to cell
1112 lysis and better access of hydrolytic enzymes from the anaerobic bacteria to the intracellular material
1113 (Cardeña et al., 2017). Ozonation pretreatment leads to significant increases in the oil extraction yield
1114 up to 100% in some cases (Lin and Hong, 2013).

1115 3.2.4.2 Hydrogen peroxide

1116 H₂O₂ is an alternative oxidizing agent for the pretreatment of microalgae with a similar mechanism to
1117 ozonation. Oxidation of mixed microalgae culture with H₂O₂ leads to biomass solubilization of 23% and
1118 a 173% increase in the methane production yield corresponding to 67% biodegradability (Martín Juárez
1119 et al., 2018). Astaxanthin extractability of 54.5 % from *H. pluvialis* was possible by treatment with 2M
1120 of H₂O₂ (Haque et al., 2016).

1121 An alternative method to increase oxidation capacity is to combine the H₂O₂ with iron ions in order to
1122 induce Fenton-like reactions. The two chemicals react to create hydroxyl radicals ($\cdot\text{OH}^-$) that attack the
1123 microalgae's organic molecules. The combined use of H₂O₂ with FeSO₄ significantly increased lipid
1124 extraction efficiency from *C. vulgaris* to 78% of total lipids compared to 41% when H₂O₂ was used
1125 alone, (Steriti et al., 2014). A Fenton-like reaction with FeCl₃ as the cell disruption reagent for lipid
1126 extraction from *Chlorella* sp. KR-1, was improved by simultaneous UV-irradiation of the reaction
1127 mixture. The unaided Fenton-like reaction yielded 80% of total lipids at 0.5% H₂O₂. The UV-Fenton-
1128 like reaction experiment improved the lipid yield to 85% at a reduced 0.3% H₂O₂ consumption (Seo et
1129 al., 2015a). The substitution of H₂O₂ with the persulfate ion (S₂O₈²⁻) was also investigated. The
1130 persulfate ion reacted with Fe³⁺ to generate SO₄⁻ and S₂O₈⁻ radicals instead of the $\cdot\text{OH}^-$ radicals of the
1131 typical Fenton reaction (Seo et al., 2016). The persulfate reagent at 2mM concentration led to improved
1132 lipid extraction yield 95%, compared to 80% for 0.5% (v/v) H₂O₂ at the same pretreatment conditions.

1133 The aggressive nature of all the oxidative pretreatment reactions leads to chemical alterations of the
1134 targeted biomolecules. Most affected are the sensitive pigments like chlorophyll. The partial or complete
1135 destruction of chlorophyll in the oxidation pretreatment of microalgae samples has been reported for
1136 ozonation (Huang et al., 2014; Kamaroddin et al., 2016) and Fenton-like reaction pretreatments (Seo et
1137 al., 2015a, p.). Additionally, significant chemical changes are observed in the composition of the
1138 extracted lipids. These changes are mainly the significant increase of saturated and monounsaturated
1139 fatty acids and the reduction in polyunsaturated fatty acids. The Fenton pretreatment of *C. vulgaris*
1140 increased the saturated, monounsaturated and C18:2 fatty acids in the extracted lipid from 35 % (w/w)
1141 in the untreated sample to 80 % (w/w) after pretreatment. At the same time the content of
1142 polyunsaturated fatty acids in the cells pretreated by Fenton reaction was dramatically decreased from
1143 60 % to 20 % (w/w) (Concas et al., 2015a). In another study of *C. vulgaris* pretreated by Fenton reaction
1144 the polyunsaturated fatty acids content decreased from 21 % (w/w) to 4.75 % (w/w) (Concas et al.,
1145 2015b). Similarly, the ozonation of *C. vulgaris* decreased the concentration of saturated fatty acids to
1146 very small or non-detectable amounts, and even decreased the content of some saturated fatty acids,
1147 probably due to very severe oxidation conditions (Huang et al., 2014). The sensitivity of polyunsaturated
1148 fatty acids to oxidation was also shown in the ozonation of *D. salina* where accumulation of saturated
1149 fatty acids and even some hydrocarbons were detected in the extracted lipids (Kamaroddin et al., 2016).

1150 The reduction in chlorophyll content is considered beneficial when the isolated lipids are transformed
1151 into biodiesel as chlorophyll deactivates the esterification catalyst (Seo et al., 2015a). Equally beneficial
1152 is the reduction in polyunsaturated fatty acids and the increase in saturated, as the corresponding
1153 saturated fatty acid methyl esters (biodiesel) are more resistant to oxidation and degradation from
1154 oxygen, heat, light etc. (Concas et al., 2015a; Steriti et al., 2014). However, if the targeted products are
1155 high added value molecules such as unsaturated fatty acids (e.g. ω 3 fatty acids) pigments (e.g.
1156 chlorophyll, carotenoids) and antioxidants (e.g. astaxanthin) that are sensitive to oxidation, such
1157 pretreatment methods may be detrimental to the product quality and/or yield. If optimization of oxidative
1158 processes to minimize product degradation is not possible, they should be exclusively used for the

1159 production of bulk products such as fermentable sugars (Keris-Sen and Gurol, 2017), biogas(Martín
1160 Juárez et al., 2018) and biodiesel (Steriti et al., 2014).

1161

1162 3.3 Enzymatic methods

1163 Enzymatic treatment is even more species specific than the methods described above. Microalgal cell
1164 walls are chemically complex and may contain several carbohydrates (cellulose, hemicellulose, pectin
1165 or chitin), protein or other organic polymers such as sporopollenin and algaenan. They are also
1166 structurally complex as these polymers are often organized in layers. Cell wall structure and composition
1167 varies between species and as a result the enzymatic activities on cell walls of different species will
1168 differ. Successful enzymatic disruption of such complex structures requires combinations of the
1169 necessary enzymatic activities. Indeed, the treatment of *N. gaditana* with Alkalase -a protease with
1170 esterase activity for peptide and amino acid esters- showed only 35% protein release (Safi et al., 2017b).
1171 Although significant hydrolysis of protein can be achieved, cell wall integrity was not compromised and
1172 cell breakage was not detected, because the remaining structural components remain intact. Another
1173 disadvantage of the protease is the reduction of the released protein functionality due to their hydrolytic
1174 fragmentation (Safi et al., 2017a).

1175 Similarly, treatment of *Scenedesmus sp.* with lysozyme -an enzyme that hydrolyses cell wall
1176 peptidoglycan by specifically degrading N-acetylglucosamine- increased the lipid extraction yield by
1177 only 16.6% compared with cellulase treatment alone (Taher et al., 2014). Lysozyme treatment on *C.*
1178 *vulgaris* thinned and delaminated the outer wall and removed hair-like fibers from the cell's surface
1179 (Gerken et al., 2013). This study compared many enzymes (chitinase, chitosanase, b-glucuronidase,
1180 pectolyase, trypsin, laminarinase, lyticase, phospholipase A1, sulfatase, b-glucuronidase and sulfatase)
1181 and showed that lysozyme was the only enzyme able to increase cell permeability when applied alone.
1182 When lysozyme and sulfatase were combined the permeabilization of 96 % of the cell population was
1183 achieved. In other experiments lysozyme treatment, enabled high protein and pigments extraction from
1184 *Chlorella sp.*, *Scenedesmus sp.* and *Chlamydomonas sp.* (Al-Zuhair et al., 2017). This required a 30fold
1185 increase in the pretreatment time (16h) compared to the previous study (Taher et al., 2014).

1186 The combination of a protease with a cellulase is a typical enzymatic treatment strategy as it can target
1187 the two main cell wall components, proteins/peptides and cellulose and/or other carbohydrates. In the
1188 treatment of *C. reinhardtii* and *C. vulgaris* Alkalase and Viscozyme (a multienzyme with b-glucanase,
1189 arabinase, hemicellulase, xylanase and other activities) led to almost complete protein and carbohydrate
1190 solubilization (Mahdy et al., 2014b). Cellulase, pectinase and lysozyme were used together for lipid
1191 extraction from *Nannochloropsis sp.* with a 22% recovery yield (Chen et al., 2017). In some cases, the
1192 cellulase-protease combination performed poorly. For example, treatment of *H. pluvialis* by combined
1193 protease K and drierase (a cellulase, hemicellulase and pectinase mix) did not enhance the extraction
1194 efficiency of astaxanthin. This was attributed to the enzymes inability to attack the cell wall
1195 sporopollenin, a very recalcitrant polymer (Mendes-Pinto et al., 2001). This underlines the importance
1196 of selecting the appropriate mix of enzymatic activities according to the microalgae specie.

1197 Combination of carbohydrate hydrolyzing enzymes is another strategy for cell wall disruption. The
1198 combined use of cellulase and pectinase at 1:1 ratio and 7000 U mL⁻¹ total enzyme dosage enabled
1199 71% astaxanthin extraction (Ye et al., 2020). The combined treatment of *Nannochloropsis sp.* with
1200 Cellulyve ® 50LC (cellulase) and Feedlyve ® GMA (Endo- β -1,4-mannanase) led to a lipid extraction
1201 yield of 90% (Zuorro et al., 2016). Treatment with carbohydrase cocktails has also been performed in
1202 tandem, when the enzymes have different temperature optima simultaneous treatment would lead to
1203 denaturation and loss of some enzymatic activity. For example, treatment of mixed cultures with β -
1204 glucosidase/cellulase at 65 °C, followed by α -amylase at 95 °C and an amyloglucosidase at 55 °C yielded
1205 95% of total sugars (Shokrkar et al., 2017). A similar total sugar yield of 92% was achieved by the same
1206 treatment strategy in the case of *H. tetrachotoma* ME03 (Onay, 2019). Fermentation of the sugar
1207 hydrolysates gave approximately 90% of theoretical bioethanol yield. It is also worth noting that the
1208 enzymatic reaction sequence initially targeted the cell wall structural carbohydrates (cellulase and b-

1209 glucosidase activities) followed by the intracellular storage carbohydrates (α -amylase and
1210 amyloglucosidase activities). This enzyme specificity towards the targeted molecules could be useful in
1211 hydrolysis products separation e.g. carbohydrates and proteins. This can be achieved by sequential
1212 treatment, first with proteases to enable protein release, followed by carbohydrate hydrolysing enzymes
1213 to remove sugars from the microalgae biomass.

1214 A variation of enzymatic pretreatment is autolysis. It occurs when the microalgae cells under stress
1215 produce cell wall degrading enzymes leading to cell lysis. When *C. reinhardtii* is exposed to nitrogen
1216 depletion stress the production of autolysin is induced. Autolysin is a protease that targets proline-rich
1217 areas in *C. reinhardtii* cell wall polypeptides. Its synthesis can be enough to enable extensive cell
1218 disruption and quantitative protein and lipid release (Sierra et al., 2017). The main benefits of this
1219 strategy are the expensive enzyme redundancy and conservation of intracellular protein integrity.
1220 Another strategy to reduce the high cost of enzymes is their *in-situ* production by coculturing the
1221 microalgae with an enzyme producing microorganism. For this reason, *C. vulgaris* was co-cultured with
1222 the bacterium *Flammeovirga yaeyamensis* that could release amylase, cellulase, and xylanase in the
1223 growth medium (Chen et al., 2013). The collected microalgae cells showed damage to the cell walls
1224 induced from the enzymes during co-culture. The supernatant from the co-culture was used to pretreat
1225 *C. vulgaris* cells in a separate pretreatment process, significantly increasing the release of hydrolyzed
1226 sugars and lipids.

1227 The non-specific enzymatic pretreatment of microalgae is also possible with the use of oxidizing
1228 enzymes. For example, manganese peroxidase (a lignin degrading enzyme using Mn^{2+} ions as oxidizing
1229 mediators) was produced by the white-rot fungi *Anthracoephyllum discolor*. The enzymatic extract was
1230 used for the pretreatment of *Botryococcus braunii* to enhance methane production (Ciudad et al., 2014).
1231 The enzymatic pretreatment increased methane production from approximately 320 mL CH_4/g VS to
1232 521 mL CH_4/g VS that corresponds to 90% of biodegradability, compared to 60% biodegradability of
1233 the untreated microalgae cells. A complex enzymatic broth containing laccase (another lignin oxidizing
1234 enzyme) from the fungus *Trametes versicolor* exhibited similar improvement in methane production
1235 from the anaerobic digestion of pre-treated mixed microalgae cultures (Hom-Diaz et al., 2016).

1236 Despite the promising results, high enzyme cost is the major hurdle to be overcome for application of
1237 enzymatic processes to biorefineries. Several strategies have been employed towards this end, such as
1238 co-cultivation and autolysis that were discussed above. Another strategy concerns the optimization of
1239 the enzyme production process to reduce production cost through increased productivity and yield.
1240 Reduction of cost through lowering the enzyme dosage has also been achieved by optimizing the
1241 enzymatic cocktail and by discovery of new more potent enzymes. Such approaches in bioethanol
1242 production from lignocellulosic biomass have reduced the enzyme production costs by a factor of ten
1243 (10) (Dahiya, 2020).

1244 Table 9

1245

1246 3.4 Novel downstream processing concepts

1247 3.4.1 Combinations of cell disruption methods

1248 Combinations of disruption methods have been frequently investigated to increase pretreatment
1249 efficiency. One example is the combination of heat treatment together with High Pressure
1250 Homogenization. This led to an initial increase in viscosity due to increased solubilization of polymers,
1251 such as proteins and carbohydrates, and a subsequent reduction of viscosity from the HPH treatment
1252 caused by a decrease in the molecular weight due to increased shear thinning (Bernaerts et al., 2018,
1253 2017). The combination allowed the treatment of cells at high concentrations, e.g. *Nannochloropsis* sp
1254 pastes of 20–25% solids (Olmstead et al., 2013). The thermal pretreatment increased cell wall
1255 susceptibility to mechanical disruption by HPH and improved lipid extraction yields (Halim et al., 2016).

1256 Ultrasonication is a pretreatment method with moderate efficiency, however, it can be easily combined
1257 with other methods to increase cell disruption capability. When used together with H_2O_2 oxidation a

1258 two-fold increase of *C. pyrenoidosa* cell lysis was achieved, compare to ultrasonication alone (Z. Duan
1259 et al., 2017). Combined with alkali pretreatment it could improve cell disruption, protein solubilization
1260 and protein yield from *C. sorokiniana* and *C. vulgaris*, compared to either pretreatment method on its
1261 own (Phong et al., 2018). Improved cell lysis was also observed when ultrasonication was combined
1262 with physical pretreatment methods such as pressure drop through nozzle spraying (M. Wang and Yuan,
1263 2015). When *Parachlorella kessleri* was treated with HPH low protein and carbohydrate yields of 11%
1264 and 20%, were obtained, respectively. When HPH was preceded by ultrasonication these values
1265 increased to 89% and 74%, respectively (Zhang et al., 2019a). Ultrasonication in combination with a
1266 two-phase system of methanol/K₃PO₄ solution can effectively combine cell-lysis and partition of
1267 proteins from cellular debris in one step (Phong et al., 2017). Cell lysis and recovery of protein in a
1268 single step is also possible with the combination of ultrasonication in an ionic liquid, that led to 95.0%
1269 protein recovery (Sze Ying Lee et al., 2017). Despite these interesting concepts, the major drawback of
1270 ultrasonication remains its inability to process high cell concentration cell-pastes. Ultrasonication at 400
1271 W has been used in combination with the ionic liquid cholinium 2-hydroxy-3-
1272 morpholinopropanesulfonate to extract 95% of total protein from *C. vulgaris* cells. Cell disruption and
1273 protein extraction were performed in a single step as the ionic liquid (as a 50mM aqueous buffer) was a
1274 suitable solvent for protein extraction (Lee et al., 2017). The processing time was 30 min and biomass
1275 concentration was 6 g L⁻¹ (Lee et al., 2017).

1276

1277 3.4.2 Novel cell disruption methods

1278

1279 3.4.2.1. CO₂ explosion

1280 Cell lysis via CO₂ explosion is an interesting method for microalgae pretreatment. The method includes
1281 cell pressurization with CO₂ that leads to increased intracellular gas concentration. It is followed by the
1282 rapid pressure release that causes expansion of intracellular gas and leads to cell disruption. It has already
1283 been shown to work for lipid extraction from the lipid producing yeast *Rhodotorula glutinis*, where CO₂
1284 explosion was performed at 35 bar, leading to significant cell breakage and release of intracellular lipids
1285 to the aqueous phase (Howlader et al., 2017). The method has also been used for the extraction of
1286 carotenoids and chlorophylls from *B. braunii* (Uquiche et al., 2016). Pretreatment at 21°C and 130 bar
1287 increased the pigments extraction yield 10-fold compared with non-treated cells. CO₂ explosion is
1288 similar to steam explosion, but the lack of heating reduces degradation reactions and could be a viable
1289 method for the extraction of valuable and sensitive intracellular molecules. Lately, a continuous CO₂
1290 explosion system was described that can effectively disrupt *Neochloris oleoabundans* UTEX 1185 cells.
1291 The continuous method reduced CO₂ consumption between 2-4 fold, reduced the process time 3-9 fold,
1292 and increased biomolecule yields by more than 2 fold compared to the batch method (Günerken et al.,
1293 2019). Other non-reactive gasses such as N₂ can also be used for cell lysis through explosive
1294 decompression (Simpson, 2010).

1295

1296 3.4.2.2 Electricity-based methods

1297 High voltage electric discharges (HVED) utilizes electrodes of needle-plate geometry to deliver high
1298 voltage pulses to microalgae suspensions. Electroporation of cells similar to that hown for PEF is also
1299 present in HVED treatment. HVED additionally induces thermal and mechanical effects to the cells due
1300 to cavitation and shockwave formation (Zhang et al., 2019c). Application of HVED (40 kV cm⁻¹, 4 ms)
1301 to *Nannochloropsis sp.* allowed the selective release of ionic components and small molecular weight
1302 water soluble components. Aggregation of the cells as a result of the electric discharge was also observed
1303 (Grimi et al., 2014). These findings were also confirmed in the HVED treatment of 1% w/w
1304 *Parachlorella kessleri* suspensions at similar conditions (40 kV cm⁻¹, 8 ms) that led to release of ionic
1305 cell components and carbohydrates but protein yield was only 15% w/w (Zhang et al., 2019b). For this
1306 reason, HVED was proposed as a first treatment step for the selective release of low molecular weight

1307 and water-soluble molecules, followed by organic solvent extraction of chlorophylls, carotenoids and
1308 lipids (Zhang et al., 2020). Application of HVED to *B. braunii* cell colonies allows the easy separation
1309 of the cells from their hydrocarbon extracellular matrix. Subsequently cells sink to the bottom while the
1310 hydrocarbon matrix rises to the surface of the process vessel, allowing for easy separation and collection
1311 (Guionet et al., 2019).

1312 Non-thermal plasma is another electricity-based method where a needle to plate electrode geometry is
1313 placed in an argon filled reactor. The application of high voltage (17 kV, 30 mA) between the electrodes
1314 generated an intense core plasma towards the microalgae biomass that led to 65-70% cell rupture of *N.*
1315 *gadicola* cells and 18.7% lipid extraction (Matos et al., 2019). The method decreased the content of
1316 polyunsaturated ω -3 fatty acids from 31% to 11% making it more suitable to biofuels applications.
1317 Nanowire-assisted electroporation of *Chlorella* sp. with copper oxide nanowire-modified three-
1318 dimensional copper foam electrodes (10 sec, 2V) led to complete inactivation and disruption of the cells
1319 and release of organic matter in the liquid phase (50 mg L⁻¹) with a very low energy consumption 0.014
1320 kWh kg⁻¹ (Bai et al., 2019). Osmotic shock of *A. platensis* at the same pretreatment conditions led to the
1321 highest lipid yield (8.9% extracted with chloroform/methanol 1:2 v/v) compared to ultrasonication,
1322 autoclave, acid and alkaline treatments (Sumprasit et al., 2017)

1323

1324 3.4.2.3 Osmotic shock

1325 Osmotic shock is a simple and effective microalgae pretreatment method. When microalgae cells are
1326 suspended in high concentration salt solutions, the density difference between cytoplasm and salt
1327 solution creates an osmotic pressure across the cellular membrane that can lead to cell disruption.
1328 Treatment of *C. vulgaris* cells in a 10% w/v NaCl solution for 48 h yielded 86% of total lipids.
1329 Microscopic observations showed that osmotic shock acts by creating holes in the cell membranes and
1330 does not lead to complete cell disintegration. The disruption effect treatment is also more homogeneous
1331 in the cell population (Heo et al., 2017). Effectiveness of the osmotic shock treatment depends on the
1332 concentration of the salt solution. When 6% w/v solutions of NaCl and sorbitol were used for the
1333 pretreatment of *C. reinhardtii*, lipid yields were not increased compared to the untreated cells (Yoo et
1334 al., 2012). When cell wall-free mutants were treated at the same conditions, lipid yield for sorbitol and
1335 NaCl increased to 20% and 35%, respectively (Yoo et al., 2012). The type and concentration of salts,
1336 incubation time and concentration of biomass are all important factors affecting the efficiency of the
1337 method (Krishna Koyande et al., 2020). Osmotic shock has been used for extraction of protein from *C.*
1338 *vulgaris* biomass. Treatment with magnesium sulphate at 100 g L⁻¹ for 10 min resulted in a high (> 90%)
1339 protein recovery. Increasing the biomass concentration from 0.25 % w/w to 5 % w/w resulted in a slight
1340 decrease of protein yield to approximately 80% (Krishna Koyande et al., 2020). Osmotic shock is an
1341 attractive cell disruption method due to its simplicity and promising yields in biomolecules extraction.
1342 Although prohibitively high treatment times of 48h are often reported, low treatment times of 10 min
1343 were also shown, indicating that optimization of the method can reduce treatment time to more realistic
1344 levels.

1345

1346 3.4.2.4 Ionic liquids

1347 Ionic liquids are organic salts with low melting points below 100 °C. The cation contains heteroatoms
1348 in the organic structure, usually nitrogen. The cations can be imidazolium, pyridinium, ammonium,
1349 cholinium and phosphonium, among others (Orr et al., 2016). The anions can be either organic or
1350 inorganic. Many ionic liquids are found in liquid form at room temperature and are very stable
1351 chemically and thermally. (Nitsos et al., 2013). These useful properties have classified them as green
1352 solvents. Ionic liquids have the ability to form hydrogen bonds, which allows them to interact with
1353 biopolymers such as cellulose found in the cell wall of microalgae. The recalcitrance of cellulose is
1354 based on the large number of hydrogen bonds formed between the cellulose microfibrils. The hydrogen
1355 bond capacity of ionic liquids allows the disruption of these interfibrillar hydrogen bonds of cellulose
1356 and can dissolve the cellulose structure. This leads to compromised microalgae cell wall structures and

1357 allows the more efficient extraction of intracellular or cell wall components of the microalgae (Chen et
1358 al., 2018). The chemical hydrolysis of cellulose by ionic liquids has also been proposed (van Spronsen
1359 et al., 2011). Interaction with other cell wall components like hemicelluloses and proteins is also
1360 possible, although the complexity of the cell wall composition and variation between species make the
1361 exact mechanism of action still unclear (Orr et al., 2016).

1362 Imidazolium based ionic liquids are very commonly used for microalgae pretreatment and are very
1363 effective at improving lipid extraction (Orr et al., 2016). For example, pretreatment of *C. vulgaris* with
1364 different types of imidazolium based ionic liquids (Table 10) led to complete cell lysis and extraction
1365 yields ranging from 45 to 98% of total lipids (Teixeira, 2012). However, employment of high
1366 temperature (140°C) for 30 min increases the energy input of the method. The requirement of heating to
1367 relatively elevated temperatures is relatively common in ionic liquid treatments, which could potentially
1368 render them less energy efficient (Orr et al., 2016). For this reason, microalgae treatment with ionic
1369 liquids at room temperatures has attracted some attention. Disruption of *C. vulgaris* cells was achieved
1370 with a variety of ionic liquids at ambient temperature for 16 h. Extraction of lipids from the pretreated
1371 cells with hexane showed significant improvement compared to untreated biomass (5% g g⁻¹ biomass
1372 lipid extraction yield). The best result (25 % g g⁻¹ biomass lipid extraction) was shown for a
1373 phosphonium ionic liquid tributylmethylphosphonium propanoate (Orr et al., 2016). These results
1374 however were performed with freeze dried biomass, that can facilitate the easier extraction of lipids due
1375 to structural damage to the cell membranes. Depending on pretreatment conditions, milder effects of
1376 ionic liquids on the lipid extraction yields from wet microalgal biomass (20%-40% of total oils)
1377 compared to freeze dried biomass (80-90 % of total oils) have been observed (Zhang et al., 2018). In the
1378 same study optimization of the pretreatment and extraction process using the phosphonium salt
1379 tributylmethylphosphonium propanoate could increase the oil yield at 80% (Zhang et al., 2018). The use
1380 of butyrolactam, caprolactam, propylammonium and hydroxypropylammonium based ionic liquids in
1381 the treatment of wet *Chlorella sp.* and *Chlorococcum sp.* achieved very good cell disruption. The best
1382 results were demonstrated by butyrolactam hexanoate with efficiency of 76% and 84% for *Chlorella sp.*
1383 and *Chlorococcum sp.*, respectively (Shankar et al., 2017). These results were obtained at room
1384 temperature, but long treatment times of 24 hours were required. Pigment extraction has also been
1385 reported with ionic liquids. Extraction of 85% of total astaxanthin was achieved with the treatment of
1386 *H. pluvialis* with a 40% w/w aqueous solution of 1-Butyl-3-Methylimidazolium Chloride for 1 h at 60
1387 °C. Astaxanthin was extracted with methanol (Liu et al., 2018).

1388 Table 10.

1389

1390 3.4.2.5 Viral cell lysis

1391 A very novel approach, appealing for its low energy requirement is the viral lysis of microalgae cell
1392 walls. For example, infection of *Chlorella variabilis* by the *Chlorella-virus Paramecium bursaria* led
1393 to almost complete cell lysis and lipid yields comparable to microwave and ultrasonication (Kim and
1394 Kim, 2018). The method also proved successful in the production of bioethanol from starch-
1395 accumulating *C. variabilis* when combined with amylase treatment of the released starch and
1396 fermentation (Cheng et al., 2013). Viral production and cell lysis efficiency was significantly influenced
1397 by the carbon to nitrogen ratio of the culture when viral infection was done *in situ* (Y.-S. Cheng et al.,
1398 2015). This approach allows for nitrogen content manipulation to optimize lipid or carbohydrate
1399 production and hence, cell lysis. Additionally, it reduces the processing times of viral cell lysis (Kim and
1400 Kim, 2018).

1401

1402 3.4.2.6 Cell disruption by contact

1403 Cell disruption of *Aurantiochytrium sp.* was achieved with a poly-dimethylaminomethylstyrene coated
1404 nylon membrane. The membrane was submerged in a culture of microalgae cells and the culture was
1405 shaken bringing the membrane and cells into contact. Contact of the negatively charged cell surface with

1406 the functionalized membrane's positive charged surface led to electrostatic interactions -possibly
1407 rearrangements in the phospholipid bilayer- and cell lysis. Although cell disruption efficiency was
1408 relatively low (26%) the method is very promising due to its simplicity and low energy requirements.
1409 (Yoo et al., 2013).

1410 A similar effect was noticed when *C. vulgaris* cells were contacted with silver nanoparticles (Abdul
1411 Razack et al., 2016). Silver nanoparticles are known for their bacteriocidal properties that are promoted
1412 by a positive surface charge (Abbaszadegan et al., 2015), and therefore a similar cell lysis mechanism
1413 may be in action. The proposed method, however, included drying of the cells both pre and post
1414 treatment and its efficiency on wet microalgae could vary.

1415

1416 3.4.2.7 Combined harvesting and cell disruption

1417 Among the most promising novel approaches are those combining harvesting with microalgae biomass
1418 pretreatment. Such methods if properly optimized can have significant benefits for both capital
1419 investment and operating costs as they reduce the number of downstream unit operations required. One
1420 such example is electroflotation, by alternating current (Florentino de Souza Silva et al., 2014).
1421 Introduction of the alternating current that breaks water molecules and creates oxidative species like O₃,
1422 H₂O₂ and -OH. These in turn cause cell disruption through oxidative reactions. The method achieved
1423 very high harvesting efficiency of 99% at 140 min processing time. Lipid extraction yield with
1424 chloroform-methanol increased from 5% in untreated cells to 25% when pretreated with electroflotation.
1425 In a similar approach ozonoflotation, combines the harvesting effect of gas bubbles with the oxidative
1426 pretreatment induced by the contact of ozone with the microalgae cell walls. The method could harvest
1427 80% of the algal biomass and double the lipids yield (measured by extraction with chloroform-methanol
1428 2:1 v/v from lyophilized biomass), indicating significant cell disruption effects (Velasquez-Orta et al.,
1429 2014). Ozonation could potentially achieve the simultaneous disinfection, harvesting and pretreatment
1430 of cultures as was demonstrated by the short time *in situ* ozonation of *D. salina* (Kamaroddin et al.,
1431 2016). The study showed that ozonation of a mixed culture of the green alga *D. salina* and *Halomonas*,
1432 a Gram-negative bacterium representing bacterial contamination, with 8 mg L⁻¹ of ozone for 10 min
1433 resulted 93% sterilization efficiency without harming the microalgae cells (Kamaroddin et al., 2016).
1434 Ozone-flotation has been used for efficient harvesting and pretreatment of microalgae for biooil
1435 production through hydrothermal liquefaction (Nava Bravo et al., 2019). One of the major disadvantages
1436 of open pond systems is the high contamination from bacteria and other microorganisms as they are
1437 exposed to the natural elements. This problem can be enhanced when process water -that often carries a
1438 high bacterial load- is used as the culture medium. Reduction of bacterial load during cultivation or
1439 during harvesting and pretreatment with ozonation can make open pond systems more attractive for
1440 microalgae biorefineries.

1441 Combined harvesting and pretreatment can also be achieved by a flocculation variation. Cells are
1442 harvested by coagulation-flocculation with ferric chloride at 200 ppm and pH 3 with 90% efficiency
1443 after 20 min. In the second step, cells are pretreated with 0.5% H₂O₂ at 90 °C. Interaction of ferric ions
1444 with H₂O₂ forms a Fenton-like reagent with potent oxidation radicals leading to 80% lipid extraction
1445 efficiency. With oxidized pyrite (20 mM) as catalyst, 90% lipid extraction and esterification were
1446 achieved (Seo et al., 2015b). As has been described in the oxidative pretreatment section, such methods
1447 may not be suitable for the valorization of antioxidants, pigments, and unsaturated fatty acids due to the
1448 severe influence of the oxidative reagents.

1449

1450 3.5 Energy efficiency of downstream processing

1451

1452 Among the reviewed microalgae harvesting methods, dissolved air flotation (Niaghi et al., 2015) and
1453 electroflocculation (Vandamme et al., 2011) appear to be the most energy efficient with 0.0025 kWh m⁻³
1454 and 0.15-1 kWh m⁻³ of sample, respectively. However, as these harvesting methods were performed at

1455 the laboratory scale, upscaling could alter the energy requirements of the processes. Centrifugation also
1456 shows a favorable energetic assessment with an energy range of 0.70-1.30 kWh m⁻³ required at high
1457 scale (Fasaei et al., 2018). The main advantage of centrifugation systems is their ability to deliver high
1458 harvesting efficiency (up to 99%) and high solids concentrations (up to 20%) at this energy range (Table
1459 11). This makes centrifugation ideal as a dewatering process able to produce microalgae pastes. Its
1460 combination with other harvesting techniques therefore could establish efficient harvesting and
1461 dewatering operations (Fasaei et al., 2018). A scenario of combined membrane filtration and
1462 centrifugation for harvesting and dewatering required 0.84 kWh·kg⁻¹, which is inside the energy input
1463 range of centrifugation alone (Table 11). Flocculation combined with centrifugation in the same scenario
1464 considerably reduced the energy requirements to 0.06 kWh·kg⁻¹ (Fasaei et al., 2018). A similar energy
1465 input (0.05 kWh·kg⁻¹) was required for combined flocculation and membrane filtration. From these
1466 examples it is apparent that size increase induced through the formation of flocks greatly enhances the
1467 efficiency of both centrifugation and membrane filtration. On the other hand, the use of flocculants
1468 increases the cost to 0.97 € Kg⁻¹ compared to the 0.30 € Kg⁻¹ required for combined membrane filtration
1469 and centrifugation. Such scenarios provide good benchmarks for development of harvesting and
1470 dewatering systems tailored to the needs of specific microalgae biorefineries. Selection of the
1471 appropriate methods could depend on the application, for example value of isolated biomolecules and
1472 the energy content of the biomass for biofuel production.

1473 The energy efficiency of High Pressure Homogenization cell disruption method has been extensively
1474 studied. Energy consumption is dependent on biomass concentration. Low cell densities clearly do not
1475 favor the treatment in terms of energy requirements. For example, 20 kWh kg⁻¹ DW is required for cell
1476 disintegration of 1.2%, w/w *C. vulgaris* suspensions (Carullo et al., 2018). Increasing the concentration
1477 of *Desmodesmus* sp. F51413 from 2.0 g L⁻¹ to 90 g L⁻¹ reduced the specific energy consumption from
1478 413 kJ g⁻¹ to 9.19 kJ g⁻¹ (Xie et al., 2016). This solids content increase does not affect carotenoid
1479 extraction yields that remains unchanged throughout the whole 2.0 g L⁻¹ to 90 g L⁻¹ range. Efficient
1480 disruption (>95%) of a 10% suspension of *N. gaditana* could also be achieved at high energy efficiency
1481 (<0.5 kWh kg⁻¹ biomass). The method is also cost efficient with a reported price range of 0.15–0.25 €
1482 kg⁻¹ of isolated protein (Safi et al., 2017a). Energy requirements are also dependent on cell wall
1483 robustness. The energy required for the disruption of single of *T. suecica* cells was 5.88 x 10⁻⁵ J cell⁻¹
1484 for *Chlorococcum* sp., it was 6.43 10⁻⁵ J cell⁻¹ (Halim et al., 2013). The cell walls of *T. suecica* are made
1485 up from polysaccharides such as mannans, together with 2-keto sugars and glycoproteins.
1486 *Chlorococcum* sp. has a cell wall primarily of polysaccharides such as cellulose pectins, hydroxyproline-
1487 rich glycoproteins and arabinogalactan proteins. Correlation of cell wall strength to compositional
1488 differences are not easy. However, the authors have attributed the difference in energy disruption
1489 requirements to the thinner cell walls of *T. suecica*. For relatively fragile microalgae like
1490 *Nannochloropsis* sp. cell disruption and energy efficiency were independent of cell density up to 25%
1491 solids (Yap et al., 2015); although this effect could be related with the ease of cell wall disruption it
1492 remains to be investigated. Treatment of the relatively tough *B. braunii* was also energy efficient (~0.04
1493 kWh kg⁻¹) when extraction of the hydrocarbon rich extracellular matrix was considered (Tsutsumi et
1494 al., 2017).

1495 Bead milling was less energy efficient compared to HPH for the disruption of *N. gaditana* cells for
1496 maximum protein release (0.43 kWh kg⁻¹ and 0.32 kWh kg⁻¹, respectively) (Safi et al., 2017a). Specific
1497 energy requirements were, reported for maximum protein release by bead milling from *T. suecica* (0.49
1498 kWh kg⁻¹), *Neochloris oleoabundans* (0.55 kWh kg⁻¹) and *C. vulgaris* (0.72 kWh kg⁻¹), with experiments
1499 performed at 9% (w/v) solids (Postma et al., 2017). It was also shown that energy efficiency can increase
1500 with the reduction of bead diameter, probably due to more efficient bead impacting. Maximum
1501 carbohydrate release was achieved at similar specific energy for *T. suecica* (0.48 kWh kg⁻¹) and *C.*
1502 *vulgaris* (0.72 kWh kg⁻¹) but higher energy input was required for *N. oleoabundans* (0.94 kWh kg⁻¹).
1503 These values indicate that both HPH and bead milling can perform below the maximum specific energy
1504 limit of 0.682 kWh kg⁻¹ for efficient target molecules extraction in the biorefinery, as set by the National
1505 Algal Biofuels Technology Roadmap target of the US Department of Energy (Postma et al., 2017). Bead
1506 milling treatment of *B. braunii* is also energy efficient (~0.08 kWh kg⁻¹).

1507 PEF pretreatment of 10% solids of *N. gaditana* cells was significantly less efficient compared to HPH
1508 and bead milling requiring an energy input of 10.42 kWh.kg⁻¹ for a very low 10% protein yield,
1509 compared to 50% with the other methods at much lower energy inputs (Safi et al., 2017a). The very
1510 small size of *N. gaditana* cells (2–4 µm) could adversely affect PEF efficiency and PEF should be only
1511 considered for larger microalgae cells. Protein extraction from *C. reinhardtii* by PEF pretreatment was
1512 equally inefficient in terms of energy input. Even at high energy input of 26.5 kWh kg⁻¹ protein yields
1513 did not exceed 15% (T Lam et al., 2017). The average cell size of *C. reinhardtii* was 4-5 µm. In contrast
1514 disruption of the cell wall deficient mutant of *C. reinhardtii*, in the same study, was more energy efficient
1515 with 40% protein yield at 2.5 kWh kg⁻¹ (T Lam et al., 2017). This shows that the cell wall of *C. reinhardtii*
1516 causes its resilience to PEF and indicates that PEF may be less suitable for microalgae species with a
1517 recalcitrant cell wall. Even at low biomass concentrations (4 g L⁻¹) PEF is still not as energy efficient as
1518 HPH and bead milling. Contradicting these results efficient extraction of molecules from *A.*
1519 *protothecoides* with PEF at 10% solids was reported with a 0.42 kWh kg⁻¹ specific energy input (Eing
1520 et al., 2013). Average cell size was larger at 7 µm and the studied microalgae is known to have a rigid
1521 cell wall consisting of a triple layer with an acetolysis-resistant material sandwiched between
1522 polysaccharides (Kalina et al., 1993). These discrepancies demonstrate how careful optimization may be
1523 needed for a more conclusive evaluation of a process potential, and that process efficiency can be species
1524 specific.

1525 Hydrothermal treatment methods also appear to be energy efficient. An 87% disruption of *N. oculata*
1526 cells could be achieved with water bath heating at 0.01 kWh L⁻¹ energy input (McMillan et al., 2013).
1527 Steam explosion of *C. sorokiniana* was very efficient as it enabled microalgal biomass solubilization in
1528 the range 65-73% with an energy input of 0.03-0.06 kWh kg⁻¹ of volatile solids (Córdova et al., 2018).
1529 These studies, however, lacked information on solids content of the treated sample and therefore the
1530 more relevant specific energy input per unit biomass could be significantly higher. An energy input of
1531 1.58 kWh kg⁻¹ for the autoclaving of *S. obliquus* (5 min at 121 °C) is a more accurate estimation of the
1532 energy requirements of hydrothermal treatment (Ansari et al., 2018). Even at these relatively mild
1533 pretreatment conditions five-fold higher energy is required compared to HPH and bead milling. Even
1534 higher energy input (2.26 kWh kg⁻¹) was reported for the efficient disruption of *Nannochloropsis*
1535 *oceanica* at 10% solids by steam explosion. At these optimal pretreatment conditions extensive cell
1536 disruption and almost complete lipid recovery could be achieved (J. Cheng et al., 2015). Although the
1537 energy input is higher compared with previously discussed HPH and bead milling significant heat
1538 recovery is possible for hydrothermal and steam explosion pretreatment methods via heat exchange
1539 (Chen et al., 2019b).

1540 Ultrasound and osmotic shock treatments were in the same energy range as hydrothermal with 1.5 kWh
1541 kg⁻¹ and 1.72 kWh kg⁻¹, respectively (Ansari et al., 2018). Enzymatic treatments are also very attractive
1542 and promising methods due to their low energy requirements. The treatment of a 10% (w/v) slurry of *N.*
1543 *gaditana* with Alkalase could be performed at a specific energy input of 0.34 kWh kg⁻¹ (Safi et al.,
1544 2017a). Although soluble protein yield was lower compared to HPH and bead milling in the same study,
1545 enzymatic reaction optimization and use of complementary enzymatic activities can greatly improve the
1546 reaction efficiency, as was previously discussed.

1547

1548 Table 11

1549 Table 12

1550

1551 4. Conclusion and outlook

1552 Although a significant amount of research on microalgae valorization has been conducted it cannot be
1553 considered exhaustive. The large number of available (as well as yet unexplored) microalgae species
1554 combined with the variety of downstream processing methods requires an even greater research and
1555 optimisation effort. A unification and standardization of reporting would allow the easier comparison of

1556 different studies and a more comprehensive understanding of the field. For example, the microalgae cell
1557 concentration in slurries is reported in various ways including number of cells per unit of volume,
1558 volatile solids per unit of volume and total solids per unit of volume, often hampering comparison of
1559 reported results between studies. Similarly, the pretreatment method efficiency has been variably
1560 evaluated by cell lysis, turbidity and product extraction yield. Between these methods cell counting is
1561 an objective way of reporting cell disruption efficiency. Turbidity is affected by cell size and
1562 agglomeration of cells or debris, and yield can be affected by the formation of degradation products
1563 (Spiden et al., 2013). Decrease of the cells dry weight (due to release of intracellular materials) and
1564 increase of free proteins and DNA in the supernatant are good indicators that the cell wall structure is
1565 compromised. These methods could be sufficient to express pretreatment efficiency, especially as partial
1566 cell wall disruption is not visible by cell counting (Safi et al., 2017a).

1567 Energy efficiency is reported as energy input per unit of microalgal biomass, energy input per unit of
1568 harvested product, energy input per unit energy content of the microalgal biomass or its lipid products.
1569 The latter calculation methods are more important when biofuels and energy are the main microalgal
1570 biorefinery target products, as the overall process net energy ratio cannot exceed the energy content of
1571 the microalgae and its products. When the production of high value molecules such as antioxidants,
1572 pigments, and food supplements is the main goal of the biorefinery energy input is more related to
1573 process cost and therefore should be reported as energy input per unit mass of the biomass.

1574 The effect of biomass concentration on the energy and the extraction efficiency of the various
1575 pretreatment methods, has not been thoroughly investigated yet. Few methods have demonstrated
1576 efficient cell disruption at truly high biomass concentrations such as 20%. Much of the works were
1577 conducted at cell concentrations around or below 10%. Indeed, it appears that 10% solids content
1578 represents a limit above which mixing, and mass transfer is not efficient in the valorization of
1579 lignocellulosic biomass (Weiss et al., 2019). Determining the concentration limits for cell disruption
1580 methods is of critical importance to select operational conditions where energy input is minimal without
1581 compromising the extraction efficiency. This limit should also be correlated to the requirements of the
1582 harvesting method in order to reduce the energy input of biomass concentration and dewatering. In this
1583 respect, energy intensive dewatering operations to very high solids content may only be required for
1584 applications of microalgae for direct use as feed or as food supplements in the form of pills and powders.

1585 Harvesting and dewatering of microalgae biomass can become more efficient by combination of
1586 methods. Initial preconcentration by simple and low-cost methods such as gravitation settling can be
1587 extremely beneficial in terms of cost and energy requirements. Therefore, selection of suitable
1588 microalgae strains with high sedimentation velocities and ability to accumulate the desired biomolecules
1589 is paramount. The initial microalgae species selection can provide considerable cost, energy and time
1590 savings, as these methods are much more efficient when applied to moderately weak cell walls.
1591 Flocculation and membrane filtration are also promising preconcentration methods and combined with
1592 efficient dewatering processes such as centrifugation can reduce energy and cost requirements. The
1593 effect of upscaling of the harvesting and dewatering operations on energy efficiency also has to be
1594 considered.

1595 Among emerging microalgae pretreatment methods thermal such as hydrothermal and steam explosion,
1596 dilute acid and alkaline hydrolysis are interesting due to the ability for fast and efficient cell disruption
1597 and fractionation of biomass components. Optimization and easy correlation of yield and energy
1598 requirements with process parameters such as time, temperature and pH with a single severity factor is
1599 also attractive. Viscosity reduction through hydrolytic reactions of macromolecules can enable such
1600 methods to handle increased solids concentrations of microalgal biomass. Energy recovery through heat
1601 exchange is an additional benefit. Enzymatic pretreatment is very promising due to the low energy
1602 requirements selectivity and the non-destructive nature of the process that can preserve the bioactive
1603 properties of the isolated molecules. Explosive decompression of microalgal cells pressurized by a gas
1604 such as CO₂ and N₂ is also interesting, although it has not been yet extensively studied. The mode of
1605 action is similar to steam explosion, but the lack of heat may make the method viable for the isolation
1606 of sensitive molecules such as pigments and antioxidants.

1607 Among the most interesting methods, however, are those combining harvesting with pretreatment such
1608 as electroflocculation, ozonoflotation, and chemical flocculation with iron and oxidation through Fenton
1609 reaction by addition of H₂O₂. Even if these methods fail to reach industrial maturity, the reduction of
1610 unit operation can provide significant benefits in terms of investment and operating costs. Similar
1611 strategies may prove critical for the implementation of a mature microalgae biorefinery in the future.

1612

1613 CRediT author statement

1614 **Christos Nitsos:** Investigation, Writing - Original Draft, Visualization, **Rayen Filali:**
1615 Conceptualization, Writing - Review & Editing, Visualization, **Behnam Taidi:**
1616 Conceptualization, Writing - Review & Editing, Supervision, **Julien Lemaire:**
1617 Conceptualization, Writing - Review & Editing

1618

1619 Acknowledgments

1620 This study was carried out in the Centre Européen de Biotechnologie et de Bioéconomie
1621 (CEBB), supported by Région Grand Est, Département de la Marne, Grand Reims and the
1622 European Union. Authors are grateful to Pr. PERRE Patrick, director of the Chair of Biotechnology of
1623 CentraleSupélec for funding all the works of this article.

1624

1625 References

- 1626 Abbaszadegan, A., Ghahramani, Y., Gholami, A., Hemmateenejad, B., Dorostkar, S.,
1627 Nabavizadeh, M., Sharghi, H., 2015. The Effect of Charge at the Surface of Silver
1628 Nanoparticles on Antimicrobial Activity against Gram-Positive and Gram-Negative
1629 Bacteria: A Preliminary Study [WWW Document]. *J. Nanomater.*
1630 <https://doi.org/10.1155/2015/720654>
- 1631 Abdel-Raouf, N., Al-Homaidan, A.A., Ibraheem, I.B.M., 2012. Microalgae and wastewater
1632 treatment. *Saudi J. Biol. Sci.* 19, 257–275. <https://doi.org/10.1016/j.sjbs.2012.04.005>
- 1633 Abdul Razack, S., Duraiarasan, S., Mani, V., 2016. Biosynthesis of silver nanoparticle and its
1634 application in cell wall disruption to release carbohydrate and lipid from *C. vulgaris*
1635 for biofuel production. *Biotechnol. Rep.* 11, 70–76.
1636 <https://doi.org/10.1016/j.btre.2016.07.001>
- 1637 Acién, F.G., Molina, E., Reis, A., Torzillo, G., Zittelli, G.C., Sepúlveda, C., Masojídek, J.,
1638 2017. 1 - Photobioreactors for the production of microalgae, in: Gonzalez-Fernandez,
1639 C., Muñoz, R. (Eds.), *Microalgae-Based Biofuels and Bioproducts*, Woodhead
1640 Publishing Series in Energy. Woodhead Publishing, pp. 1–44.
1641 <https://doi.org/10.1016/B978-0-08-101023-5.00001-7>
- 1642 Adesanya, V.O., Cadena, E., Scott, S.A., Smith, A.G., 2014. Life cycle assessment on
1643 microalgal biodiesel production using a hybrid cultivation system. *Bioresour. Technol.*
1644 163, 343–355. <https://doi.org/10.1016/j.biortech.2014.04.051>
- 1645 Akao, P.K., Cohen-Yaniv, V., Peretz, R., Kinel-Tahan, Y., Yehoshua, Y., Mamane, H., 2019.
1646 Effect of ozonation on *Spirulina platensis* filaments by dynamic imaging particle
1647 analysis. *Biomass Bioenergy* 127, 105247.
1648 <https://doi.org/10.1016/j.biombioe.2019.05.016>
- 1649 Alhattab, M., Brooks, M.S.-L., 2017. Dispersed air flotation and foam fractionation for the
1650 recovery of microalgae in the production of biodiesel. *Sep. Sci. Technol.* 52, 2002–
1651 2016. <https://doi.org/10.1080/01496395.2017.1308957>

1652 Alhattab, M., Kermanshahi-pour, A., Su-Ling Brooks, M., 2019. Dispersed air flotation of
1653 *Chlorella saccharophila* and subsequent extraction of lipids – Effect of supercritical
1654 CO₂ extraction parameters and surfactant pretreatment. *Biomass Bioenergy* 127,
1655 105297. <https://doi.org/10.1016/j.biombioe.2019.105297>

1656 Al-Zuhair, S., Ashraf, S., Hisaindee, S., Darmaki, N.A., Battah, S., Svistunenکو, D., Reeder,
1657 B., Stanway, G., Chaudhary, A., 2017. Enzymatic pre-treatment of microalgae cells
1658 for enhanced extraction of proteins. *Eng. Life Sci.* 17, 175–185.
1659 <https://doi.org/10.1002/elsc.201600127>

1660 Amaro, H.M., Sousa-Pinto, I., Malcata, F.X., Guedes, A.C., 2017. 16 - Microalgal fatty
1661 acids—From harvesting until extraction, in: Gonzalez-Fernandez, C., Muñoz, R.
1662 (Eds.), *Microalgae-Based Biofuels and Bioproducts*, Woodhead Publishing Series in
1663 Energy. Woodhead Publishing, pp. 369–400. <https://doi.org/10.1016/B978-0-08-101023-5.00016-9>

1665 Ansari, F.A., Gupta, S.K., Nasr, M., Rawat, I., Bux, F., 2018. Evaluation of various cell
1666 drying and disruption techniques for sustainable metabolite extractions from
1667 microalgae grown in wastewater: A multivariate approach. *J. Clean. Prod.* 182, 634–
1668 643. <https://doi.org/10.1016/j.jclepro.2018.02.098>

1669 Antwi, E., Engler, N., Nelles, M., Schüch, A., 2019. Anaerobic digestion and the effect of
1670 hydrothermal pretreatment on the biogas yield of cocoa pods residues. *Waste Manag.*
1671 88, 131–140. <https://doi.org/10.1016/j.wasman.2019.03.034>

1672 Araujo, G.S., Matos, L.J.B.L., Fernandes, J.O., Cartaxo, S.J.M., Gonçalves, L.R.B.,
1673 Fernandes, F.A.N., Farias, W.R.L., 2013. Extraction of lipids from microalgae by
1674 ultrasound application: Prospection of the optimal extraction method. *Ultrason.*
1675 *Sonochem.* 20, 95–98. <https://doi.org/10.1016/j.ultsonch.2012.07.027>

1676 Araya, B., Gouveia, L., Nobre, B., Reis, A., Chamy, R., Poirrier, P., 2014. Evaluation of the
1677 simultaneous production of lutein and lipids using a vertical alveolar panel bioreactor
1678 for three *Chlorella* species. *Algal Res.* 6, 218–222.
1679 <https://doi.org/10.1016/j.algal.2014.06.003>

1680 Arun, J., 2017. Ultrasound assisted enhanced extraction of lutein (β , ϵ -carotene-3 , 3'-diol)
1681 from Microalga (*Chlorella pyrenoidosa*) grown in wastewater : Optimization
1682 through Response Surface Methodology. <https://doi.org/10.30955/gnj.002415>

1683 Azaman, S.N.A., Nagao, N., Yusoff, F.M., Tan, S.W., Yeap, S.K., 2017. A comparison of the
1684 morphological and biochemical characteristics of *Chlorella sorokiniana* and *Chlorella*
1685 *zofingiensis* cultured under photoautotrophic and mixotrophic conditions. *PeerJ* 5.
1686 <https://doi.org/10.7717/peerj.3473>

1687 Badvipour, S., Eustance, E., Sommerfeld, M.R., 2016. Process evaluation of energy
1688 requirements for feed production using dairy wastewater for algal cultivation:
1689 Theoretical approach. *Algal Res.* 19, 207–214.
1690 <https://doi.org/10.1016/j.algal.2016.08.017>

1691 Bai, Y., Huo, Z.-Y., Wu, Y.-H., Hu, H.-Y., 2019. Efficient nanowire-assisted electroporation
1692 and cellular inclusion release of microalgal cells achieved by a low voltage. *Sci. Total*
1693 *Environ.* 667, 191–196. <https://doi.org/10.1016/j.scitotenv.2019.02.337>

1694 Balasundaram, B., Skill, S.C., Llewellyn, C.A., 2012. A low energy process for the recovery of
1695 bioproducts from cyanobacteria using a ball mill. *Biochem. Eng. J.* 69, 48–56.
1696 <https://doi.org/10.1016/j.bej.2012.08.010>

1697 Balduyck, L., Bruneel, C., Goiris, K., Dejonghe, C., Foubert, I., 2018. Influence of High
1698 Pressure Homogenization on Free Fatty Acid Formation in *Nannochloropsis* sp. *Eur. J.*
1699 *Lipid Sci. Technol.* 120, 1700436. <https://doi.org/10.1002/ejlt.201700436>

1700 Baroni, É.G., Yap, K.Y., Webley, P.A., Scales, P.J., Martin, G.J.O., 2019. The effect of
1701 nitrogen depletion on the cell size, shape, density and gravitational settling of

1702 Nannochloropsis salina, Chlorella sp. (marine) and Haematococcus pluvialis. Algal
1703 Res. 39, 101454. <https://doi.org/10.1016/j.algal.2019.101454>

1704 Baudelet, P.-H., Ricochon, G., Linder, M., Muniglia, L., 2017. A new insight into cell walls
1705 of Chlorophyta. Algal Res. 25, 333–371. <https://doi.org/10.1016/j.algal.2017.04.008>

1706 Ben Amor-Ben Ayed, H., Taidi, B., Ayadi, H., Pareau, D., Stambouli, M., 2016. Magnesium
1707 Uptake by the Green Microalga Chlorella vulgaris in Batch Cultures. J. Microbiol.
1708 Biotechnol. 26, 503–510. <https://doi.org/10.4014/jmb.1507.07039>

1709 Ben Amor-Ben Ayed, H., Taidi, B., Ayadi, H., Pareau, D., Stambouli, M., 2015. Effect of
1710 magnesium ion concentration in autotrophic cultures of Chlorella vulgaris. Algal Res.-
1711 Biomass Biofuels Bioprod. 9, 291–296. <https://doi.org/10.1016/j.algal.2015.03.021>

1712 Ben Ayed, H.B.A., Taidi, Behnam, Ayadi H, Pareau D, Stambouli M, 2017. The Use of
1713 Chlorella Vulgaris to Accumulate Magnesium under Different Culture Conditions. J.
1714 Appl. Biotechnol. Bioeng. 2. <https://doi.org/10.15406/jabb.2017.02.00043>

1715 Bernaerts, T.M.M., Panozzo, A., Doumen, V., Foubert, I., Gheysen, L., Goiris, K.,
1716 Moldenaers, P., Hendrickx, M.E., Van Loey, A.M., 2017. Microalgal biomass as a
1717 (multi)functional ingredient in food products: Rheological properties of microalgal
1718 suspensions as affected by mechanical and thermal processing. Algal Res. 25, 452–
1719 463. <https://doi.org/10.1016/j.algal.2017.05.014>

1720 Bernaerts, T.M.M., Panozzo, A., Verhaegen, K.A.F., Gheysen, L., Foubert, I., Moldenaers, P.,
1721 Hendrickx, M.E., Loey, A.M.V., 2018. Impact of different sequences of mechanical
1722 and thermal processing on the rheological properties of Porphyridium cruentum and
1723 Chlorella vulgaris as functional food ingredients. Food Funct. 9, 2433–2446.
1724 <https://doi.org/10.1039/C8FO00261D>

1725 Besson, A., Formosa-Dague, C., Guiraud, P., 2019. Flocculation-flotation harvesting
1726 mechanism of Dunaliella salina: From nanoscale interpretation to industrial
1727 optimization. Water Res. 155, 352–361. <https://doi.org/10.1016/j.watres.2019.02.043>

1728 Bousquet, O., Sellier, N., Le Goffic, F., 1994. Characterization and purification of
1729 polyunsaturated fatty acids from microalgae by gas chromatography-mass
1730 spectrometry and countercurrent chromatography. Chromatographia 39, 40–44.
1731 <https://doi.org/10.1007/BF02320456>

1732 Byreddy, A.R., Barrow, C.J., Puri, M., 2016. Bead milling for lipid recovery from
1733 thraustochytrid cells and selective hydrolysis of Schizochytrium DT3 oil using lipase.
1734 Bioresour. Technol. 200, 464–469. <https://doi.org/10.1016/j.biortech.2015.10.019>

1735 Cardeña, R., Moreno, G., Bakonyi, P., Buitrón, G., 2017. Enhancement of methane
1736 production from various microalgae cultures via novel ozonation pretreatment. Chem.
1737 Eng. J. 307, 948–954. <https://doi.org/10.1016/j.cej.2016.09.016>

1738 Carullo, D., Abera, B.D., Casazza, A.A., Donsì, F., Perego, P., Ferrari, G., Pataro, G., 2018.
1739 Effect of pulsed electric fields and high pressure homogenization on the aqueous
1740 extraction of intracellular compounds from the microalgae Chlorella vulgaris. Algal
1741 Res. 31, 60–69. <https://doi.org/10.1016/j.algal.2018.01.017>

1742 Carvalho, A.P., Silva, S.O., Baptista, J.M., Malcata, F.X., 2011. Light requirements in
1743 microalgal photobioreactors: an overview of biophotonic aspects. Appl. Microbiol.
1744 Biotechnol. 89, 1275–1288. <https://doi.org/10.1007/s00253-010-3047-8>

1745 Cerón, M.C., Campos, I., Sánchez, J.F., Acien, F.G., Molina, E., Fernández-Sevilla, J.M.,
1746 2008. Recovery of lutein from microalgae biomass: development of a process for
1747 Scenedesmus almeriensis biomass. J. Agric. Food Chem. 56, 11761–11766.
1748 <https://doi.org/10.1021/jf8025875>

1749 Chatsungnoen, T., Chisti, Y., 2016. Continuous flocculation-sedimentation for harvesting
1750 Nannochloropsis salina biomass. J. Biotechnol. 222, 94–103.
1751 <https://doi.org/10.1016/j.jbiotec.2016.02.020>

- 1752 Chen, C.-Y., Bai, M.-D., Chang, J.-S., 2013. Improving microalgal oil collecting efficiency by
1753 pretreating the microalgal cell wall with destructive bacteria. *Biochem. Eng. J.* 81,
1754 170–176. <https://doi.org/10.1016/j.bej.2013.10.014>
- 1755 Chen, H., Fu, Q., Liao, Q., Xiao, C., Huang, Y., Xia, A., Zhu, X., Kang, Z., 2019a.
1756 Rheokinetics of microalgae slurry during hydrothermal pretreatment processes.
1757 *Bioresour. Technol.* 289, 121650. <https://doi.org/10.1016/j.biortech.2019.121650>
- 1758 Chen, H., Fu, Q., Liao, Q., Zhang, H., Huang, Y., Xia, A., Zhu, X., 2018. Rheological
1759 properties of microalgae slurry for application in hydrothermal pretreatment systems.
1760 *Bioresour. Technol.* 249, 599–604. <https://doi.org/10.1016/j.biortech.2017.10.051>
- 1761 Chen, H., Liao, Q., Fu, Q., Huang, Y., Xia, A., Xiao, C., Zhu, X., 2019b. Convective heat
1762 transfer characteristics of microalgae slurries in a circular tube flow. *Int. J. Heat Mass*
1763 *Transf.* 137, 823–834. <https://doi.org/10.1016/j.ijheatmasstransfer.2019.03.166>
- 1764 Chen, Q., Liu, D., Wu, C., Xu, A., Xia, W., Wang, Z., Wen, F., Yu, D., 2017. Influence of a
1765 facile pretreatment process on lipid extraction from *Nannochloropsis* sp. through an
1766 enzymatic hydrolysis reaction. *RSC Adv.* 7, 53270–53277.
1767 <https://doi.org/10.1039/C7RA11483D>
- 1768 Chen, Y.-H., Walker, T.H., 2011. Biomass and lipid production of heterotrophic microalgae
1769 *Chlorella protothecoides* by using biodiesel-derived crude glycerol. *Biotechnol. Lett.*
1770 33, 1973. <https://doi.org/10.1007/s10529-011-0672-y>
- 1771 Chen, Z., Wang, L., Qiu, S., Ge, S., 2018. Determination of Microalgal Lipid Content and
1772 Fatty Acid for Biofuel Production [WWW Document]. *BioMed Res. Int.*
1773 <https://doi.org/10.1155/2018/1503126>
- 1774 Cheng, C.-H., Du, T.-B., Pi, H.-C., Jang, S.-M., Lin, Y.-H., Lee, H.-T., 2011. Comparative
1775 study of lipid extraction from microalgae by organic solvent and supercritical CO₂.
1776 *Bioresour. Technol.* 102, 10151–10153. <https://doi.org/10.1016/j.biortech.2011.08.064>
- 1777 Cheng, J., Huang, R., Li, T., Zhou, J., Cen, K., 2015. Physicochemical characterization of wet
1778 microalgal cells disrupted with instant catapult steam explosion for lipid extraction.
1779 *Bioresour. Technol.* 191, 66–72. <https://doi.org/10.1016/j.biortech.2015.05.005>
- 1780 Cheng, J., Sun, J., Huang, Y., Zhou, J., Cen, K., 2014. Fractal microstructure characterization
1781 of wet microalgal cells disrupted with ultrasonic cavitation for lipid extraction.
1782 *Bioresour. Technol.* 170, 138–143. <https://doi.org/10.1016/j.biortech.2014.07.090>
- 1783 Cheng, Y., Zhou, W., Gao, C., Lan, K., Gao, Y., Wu, Q., 2009. Biodiesel production from
1784 Jerusalem artichoke (*Helianthus Tuberosus* L.) tuber by heterotrophic microalgae
1785 *Chlorella protothecoides*. *J. Chem. Technol. Biotechnol.* 84, 777–781.
1786 <https://doi.org/10.1002/jctb.2111>
- 1787 Cheng, Y.-S., Labavitch, J., VanderGheynst, J.S., 2015. Organic and Inorganic Nitrogen
1788 Impact *Chlorella variabilis* Productivity and Host Quality for Viral Production and
1789 Cell Lysis. *Appl. Biochem. Biotechnol.* 176, 467–479. <https://doi.org/10.1007/s12010-015-1588-0>
- 1791 Cheng, Y.-S., Zheng, Y., Labavitch, J.M., VanderGheynst, J.S., 2013. Virus infection of
1792 *Chlorella variabilis* and enzymatic saccharification of algal biomass for bioethanol
1793 production. *Bioresour. Technol.* 137, 326–331.
1794 <https://doi.org/10.1016/j.biortech.2013.03.055>
- 1795 Cheng, X., Riordon, J., Nguyen, B., Ooms, M.D., Sinton, D., 2017. Hydrothermal disruption
1796 of algae cells for astaxanthin extraction. *Green Chem.* 19, 106–111.
1797 <https://doi.org/10.1039/C6GC02746F>
- 1798 Chia, S.R., Chew, K.W., Zaid, H.F.M., Chu, D.-T., Tao, Y., Show, P.L., 2019. Microalgal
1799 Protein Extraction From *Chlorella vulgaris* FSP-E Using Triphasic Partitioning
1800 Technique With Sonication. *Front. Bioeng. Biotechnol.* 7.
1801 <https://doi.org/10.3389/fbioe.2019.00396>

1802 Choi, S.P., Nguyen, M.T., Sim, S.J., 2010. Enzymatic pretreatment of *Chlamydomonas*
1803 *reinhardtii* biomass for ethanol production. *Bioresour. Technol.* 101, 5330–5336.
1804 <https://doi.org/10.1016/j.biortech.2010.02.026>

1805 Chisti Y., Moo-Young M., Disruption of microbial cells for intracellular products, 1986,
1806 *Enzyme and Microbial Technology* 8, 194–204. [https://doi.org/10.1016/0141-](https://doi.org/10.1016/0141-0229(86)90087-6)
1807 [0229\(86\)90087-6](https://doi.org/10.1016/0141-0229(86)90087-6)

1808 Chtourou, H., Dahmen, I., Jebali, A., Karray, F., Hassairi, I., Abdelkafi, S., Ayadi, H., Sayadi,
1809 S., Dhouib, A., 2015. Characterization of *Amphora* sp., a newly isolated diatom wild
1810 strain, potentially usable for biodiesel production. *Bioprocess Biosyst. Eng.* 38, 1381–
1811 1392. <https://doi.org/10.1007/s00449-015-1379-6>

1812 Chu, S., Cui, Y., Liu, N., 2017. The path towards sustainable energy. *Nat. Mater.* 16, 16–22.
1813 <https://doi.org/10.1038/nmat4834>

1814 Ciudad, G., Rubilar, O., Azócar, L., Toro, C., Cea, M., Torres, Á., Ribera, A., Navia, R.,
1815 2014. Performance of an enzymatic extract in *Botryococcus braunii* cell wall disruption.
1816 *J. Biosci. Bioeng.* 117, 75–80. <https://doi.org/10.1016/j.jbiosc.2013.06.012>

1817 Clement-Larosiere, B., Lopes, F., Goncalves, A., Taidi, B., Benedetti, M., Minier, M., Pareau,
1818 D., 2014. Carbon dioxide biofixation by *Chlorella vulgaris* at different CO₂
1819 concentrations and light intensities. *Eng. Life Sci.* 14, 509–519.
1820 <https://doi.org/10.1002/elsc.201200212>

1821 Collet, P., Hélias, A., Lardon, L., Ras, M., Goy, R.-A., Steyer, J.-P., 2011. Life-cycle
1822 assessment of microalgae culture coupled to biogas production. *Bioresour. Technol.*,
1823 Special Issue: Biofuels - II: Algal Biofuels and Microbial Fuel Cells 102, 207–214.
1824 <https://doi.org/10.1016/j.biortech.2010.06.154>

1825 Collotta, M., Champagne, P., Mabee, W., Tomasoni, G., Leite, G.B., Busi, L., Alberti, M.,
1826 2017. Comparative LCA of Flocculation for the Harvesting of Microalgae for Biofuels
1827 Production. *Procedia CIRP*, The 24th CIRP Conference on Life Cycle Engineering 61,
1828 756–760. <https://doi.org/10.1016/j.procir.2016.11.146>

1829 Concas, A., Pisu, M., Cao, G., 2015a. Microalgal cell disruption through fenton reaction:
1830 experiments, modeling and remarks on its effect on the extracted lipids composition.
1831 *Chem. Eng. Trans.* 43, 367–372. <https://doi.org/10.3303/CET1543062>

1832 Concas, A., Pisu, M., Cao, G., 2015b. Disruption of microalgal cells for lipid extraction
1833 through Fenton reaction: Modeling of experiments and remarks on its effect on lipids
1834 composition. *Chem. Eng. J.* 263, 392–401. <https://doi.org/10.1016/j.cej.2014.11.012>

1835 Córdova, O., Passos, F., Chamy, R., 2018. Physical Pretreatment Methods for Improving
1836 Microalgae Anaerobic Biodegradability. *Appl. Biochem. Biotechnol.* 185, 114–126.
1837 <https://doi.org/10.1007/s12010-017-2646-6>

1838 Costa, G., Plazanet, I., 2016. Plant Cell Wall, a Challenge for Its Characterisation. *Adv. Biol.*
1839 *Chem.* 6, 70–105. <https://doi.org/10.4236/abc.2016.63008>

1840 Coward, T., Lee, J.G.M., Caldwell, G.S., 2014. Harvesting microalgae by CTAB-aided foam
1841 flotation increases lipid recovery and improves fatty acid methyl ester characteristics.
1842 *Biomass Bioenergy* 67, 354–362. <https://doi.org/10.1016/j.biombioe.2014.05.019>

1843 Cuellar-Bermudez, S.P., Aguilar-Hernandez, I., Cardenas-Chavez, D.L., Ornelas-Soto, N.,
1844 Romero-Ogawa, M.A., Parra-Saldivar, R., 2015. Extraction and purification of high-
1845 value metabolites from microalgae: essential lipids, astaxanthin and phycobiliproteins.
1846 *Microb. Biotechnol.* 8, 190–209. <https://doi.org/10.1111/1751-7915.12167>

1847 Cuellar-Bermudez, S.P., Kilimtzdi, E., Devaere, J., Goiris, K., Gonzalez-Fernandez, C.,
1848 Wattiez, R., Muylaert, K., 2019. Harvesting of *Arthrospira platensis* with helicoidal
1849 and straight trichomes using filtration and centrifugation. *Sep. Sci. Technol.* 0, 1–10.
1850 <https://doi.org/10.1080/01496395.2019.1624573>

- 1851 Dahiya, A. (Ed.), 2020. Chapter 28 - Reducing enzyme costs, novel combinations, and
1852 advantages of enzymes could lead to improved cost-effective biofuels' production, in:
1853 Bioenergy (Second Edition). Academic Press, pp. 573–579.
1854 <https://doi.org/10.1016/B978-0-12-815497-7.00028-2>
- 1855 Danquah, M.K., Ang, L., Uduman, N., Moheimani, N., Forde, G.M., 2009. Dewatering of
1856 microalgal culture for biodiesel production: exploring polymer flocculation and
1857 tangential flow filtration. *J. Chem. Technol. Biotechnol.* 84, 1078–1083.
1858 <https://doi.org/10.1002/jctb.2137>
- 1859 Das, P., Thaher, M., Khan, S., AbdulQuadir, M., Al-Jabri, H., 2019. The effect of culture
1860 salinity on the harvesting of microalgae biomass using pilot-scale tangential-flow-
1861 filter membrane. *Bioresour. Technol.* 293, 122057.
1862 <https://doi.org/10.1016/j.biortech.2019.122057>
- 1863 de Farias Silva, C.E., Meneghello, D., de Souza Abud, A.K., Bertucco, A., 2020. Pretreatment
1864 of microalgal biomass to improve the enzymatic hydrolysis of carbohydrates by
1865 ultrasonication: Yield vs energy consumption. *J. King Saud Univ. - Sci.* 32, 606–613.
1866 <https://doi.org/10.1016/j.jksus.2018.09.007>
- 1867 Delgadillo-Mirquez, L., Lopes, F., Taidi, B., Pareau, D., 2016. Nitrogen and phosphate
1868 removal from wastewater with a mixed microalgae and bacteria culture. *Biotechnol.*
1869 *Rep.* 11, 18–26. <https://doi.org/10.1016/j.btre.2016.04.003>
- 1870 Demirbas, M.F., Balat, M., Balat, H., 2009. Potential contribution of biomass to the
1871 sustainable energy development. *Energy Convers. Manag.* 50, 1746–1760.
1872 <https://doi.org/10.1016/j.enconman.2009.03.013>
- 1873 Depraetere, O., Pierre, G., Deschoenmaeker, F., Badri, H., Foubert, I., Leys, N., Markou, G.,
1874 Wattiez, R., Michaud, P., Muylaert, K., 2015. Harvesting carbohydrate-rich
1875 *Arthrospira platensis* by spontaneous settling. *Bioresour. Technol.* 180, 16–21.
1876 <https://doi.org/10.1016/j.biortech.2014.12.084>
- 1877 Di Serio, M., Tesser, R., Santacesaria, E., 2001. A kinetic and mass transfer model to simulate
1878 the growth of baker's yeast in industrial bioreactors. *Chem. Eng. J., FRONTIERS IN*
1879 *CHEMICAL REACTION ENGINEERING* 82, 347–354.
1880 [https://doi.org/10.1016/S1385-8947\(00\)00353-3](https://doi.org/10.1016/S1385-8947(00)00353-3)
- 1881 Doucha, J., Lívanský, K., 2008. Influence of processing parameters on disintegration of
1882 *Chlorella* cells in various types of homogenizers. *Appl. Microbiol. Biotechnol.* 81,
1883 431. <https://doi.org/10.1007/s00253-008-1660-6>
- 1884 Duan, Z., Tan, X., Dai, K., Gu, H., Yang, H., 2017. Evaluation on H₂O₂-aided ultrasonic
1885 pretreatment for cell disruption of *Chlorella pyrenoidosa*. *Asia-Pac. J. Chem. Eng.* 12,
1886 502–510. <https://doi.org/10.1002/apj.2093>
- 1887 Duan, Zhipeng, Tan, X., Guo, J., Kahehu, C.W., Yang, H., Zheng, X., Zhu, F., 2017. Effects
1888 of biological and physical properties of microalgae on disruption induced by a low-
1889 frequency ultrasound. *J. Appl. Phycol.* 29, 2937–2946.
1890 <https://doi.org/10.1007/s10811-017-1178-3>
- 1891 Eing, C., Goettel, M., Straessner, R., Gusbeth, C., Frey, W., 2013. Pulsed Electric Field
1892 Treatment of Microalgae—Benefits for Microalgae Biomass Processing. *IEEE Trans.*
1893 *Plasma Sci.* 41, 2901–2907. <https://doi.org/10.1109/TPS.2013.2274805>
- 1894 Elcik, H., Cakmakci, M., 2017. Harvesting of microalgae via submerged membranes: flux,
1895 fouling and its reversibility. *Membr. Water Treat.* 8, 499–515.
1896 <https://doi.org/10.12989/mwt.2017.8.5.499>
- 1897 Elcik, H., Cakmakci, M., Ozkaya, B., 2017. Preparation and characterisation of novel
1898 polysulfone membranes modified with Pluronic F-127 for reducing microalgal
1899 fouling. *Chem. Pap.* 71, 1271–1290. <https://doi.org/10.1007/s11696-016-0120-5>

- 1900 Fábryová, T., Cheel, J., Kubáč, D., Hrouzek, P., Vu, D.L., Tůmová, L., Kopecký, J., 2019.
 1901 Purification of lutein from the green microalgae *Chlorella vulgaris* by integrated use of
 1902 a new extraction protocol and a multi-injection high performance counter-current
 1903 chromatography (HPCCC). *Algal Res.* 41, 101574.
 1904 <https://doi.org/10.1016/j.algal.2019.101574>
- 1905 Fan, J., Zheng, L., Bai, Y., Saroussi, S., Grossman, A.R., 2017. Flocculation of
 1906 *Chlamydomonas reinhardtii* with Different Phenotypic Traits by Metal Cations and
 1907 High pH. *Front. Plant Sci.* 8. <https://doi.org/10.3389/fpls.2017.01997>
- 1908 Fasaeei, F., Bitter, J.H., Slegers, P.M., van Boxtel, A.J.B., 2018. Techno-economic evaluation
 1909 of microalgae harvesting and dewatering systems. *Algal Res.* 31, 347–362.
 1910 <https://doi.org/10.1016/j.algal.2017.11.038>
- 1911 Filali, R., Taidi, B., Pareau, D., 2019.
 1912 Optimization of a raceway pond system for wastewater treatment: a review. *Crit. Rev.*
 1913 *Biotechnol.* 39, 422–435. <https://doi.org/10.1080/07388551.2019.1571007>
- 1914 Florentino de Souza Silva, A.P., Costa, M.C., Colzi Lopes, A., Fares Abdala Neto, E., Carrhá
 1915 Leitão, R., Mota, C.R., Bezerra dos Santos, A., 2014. Comparison of pretreatment
 1916 methods for total lipids extraction from mixed microalgae. *Renew. Energy* 63, 762–
 766. <https://doi.org/10.1016/j.renene.2013.10.038>
- 1917 Fu, Q., Zhang, H., Chen, H., Liao, Q., Xia, A., Huang, Y., Zhu, X., Reungsang, A., Liu, Z.,
 1918 2018. Hydrothermal hydrolysis pretreatment of microalgae slurries in a continuous
 1919 reactor under subcritical conditions for large-scale application. *Bioresour. Technol.*
 1920 266, 306–314. <https://doi.org/10.1016/j.biortech.2018.06.088>
- 1921 García-Cubero, M.T., González-Benito, G., Indacochea, I., Coca, M., Bolado, S., 2009.
 1922 Effect of ozonolysis pretreatment on enzymatic digestibility of wheat and rye straw.
 1923 *Bioresour. Technol.* 100, 1608–1613. <https://doi.org/10.1016/j.biortech.2008.09.012>
- 1924 Garrote, G., Domínguez, H., Parajó, J.C., 1999. Hydrothermal processing of lignocellulosic
 1925 materials. *Holz Als Roh- Werkst.* 57, 191–202.
 1926 <https://doi.org/10.1007/s001070050039>
- 1927 Gayen, K., Bhowmick, T.K., Maity, S.K., Bhowmick, T.K., Maity, S.K., 2019. Sustainable
 1928 Downstream Processing of Microalgae for Industrial Application. CRC Press.
 1929 <https://doi.org/10.1201/9780429027970>
- 1930 Gerde, J.A., Montalbo-Lomboy, M., Yao, L., Grewell, D., Wang, T., 2012. Evaluation of
 1931 microalgae cell disruption by ultrasonic treatment. *Bioresour. Technol.* 125, 175–181.
 1932 <https://doi.org/10.1016/j.biortech.2012.08.110>
- 1933 Gerken, H.G., Donohoe, B., Knoshaug, E.P., 2013. Enzymatic cell wall degradation of
 1934 *Chlorellavulgaris* and other microalgae for biofuels production. *Planta* 237, 239–253.
 1935 <https://doi.org/10.1007/s00425-012-1765-0>
- 1936 Giorno, F., Mazzei, R., Giorno, L., 2013. Purification of triacylglycerols for biodiesel
 1937 production from *Nannochloropsis* microalgae by membrane technology. *Bioresour.*
 1938 *Technol.* 140, 172–178. <https://doi.org/10.1016/j.biortech.2013.04.073>
- 1939 Gonçalves, A.L., Pires, J.C.M., Simões, M., 2017. A review on the use of microalgal
 1940 consortia for wastewater treatment. *Algal Res., Wastewater and Algae; opportunities,*
 1941 *challenges and long term sustainability* 24, 403–415.
 1942 <https://doi.org/10.1016/j.algal.2016.11.008>
- 1943 Granados, M.R., Acién, F.G., Gómez, C., Fernández-Sevilla, J.M., Molina Grima, E., 2012.
 1944 Evaluation of flocculants for the recovery of freshwater microalgae. *Bioresour.*
 1945 *Technol.* 118, 102–110. <https://doi.org/10.1016/j.biortech.2012.05.018>
- 1946 Greenly, J.M., Tester, J.W., 2015. Ultrasonic cavitation for disruption of microalgae.
 1947 *Bioresour. Technol., Advances in biofuels and chemicals from algae* 184, 276–279.
 1948 <https://doi.org/10.1016/j.biortech.2014.11.036>

- 1949 Grimi, N., Dubois, A., Marchal, L., Jubeau, S., Lebovka, N.I., Vorobiev, E., 2014. Selective
 1950 extraction from microalgae *Nannochloropsis* sp. using different methods of cell
 1951 disruption. *Bioresour. Technol.* 153, 254–259.
 1952 <https://doi.org/10.1016/j.biortech.2013.12.011>
- 1953 Gügi, B., Le Costaouec, T., Burel, C., Lerouge, P., Helbert, W., Bardor, M., 2015. Diatom-
 1954 Specific Oligosaccharide and Polysaccharide Structures Help to Unravel Biosynthetic
 1955 Capabilities in Diatoms. *Mar. Drugs* 13, 5993–6018.
 1956 <https://doi.org/10.3390/md13095993>
- 1957 Guionet, A., Oura, K., Akiyama, H., Hosano, H., 2019. The narrow window of energy
 1958 application for oil extraction by arc discharge. *J. Appl. Phycol.* 31, 89–96.
 1959 <https://doi.org/10.1007/s10811-018-1579-y>
- 1960 Guldhe, A., Misra, R., Singh, P., Rawat, I., Bux, F., 2016. An innovative electrochemical
 1961 process to alleviate the challenges for harvesting of small size microalgae by using
 1962 non-sacrificial carbon electrodes. *Algal Res.* 19, 292–298.
 1963 <https://doi.org/10.1016/j.algal.2015.08.014>
- 1964 Guldhe, A., Singh, B., Rawat, I., Ramluckan, K., Bux, F., 2014. Efficacy of drying and cell
 1965 disruption techniques on lipid recovery from microalgae for biodiesel production. *Fuel*
 1966 128, 46–52. <https://doi.org/10.1016/j.fuel.2014.02.059>
- 1967 Günerken, E., D’Hondt, E., Eppink, M.H.M., Garcia-Gonzalez, L., Elst, K., Wijffels, R.H.,
 1968 2015a. Cell disruption for microalgae biorefineries. *Biotechnol. Adv.* 33, 243–260.
 1969 <https://doi.org/10.1016/j.biotechadv.2015.01.008>
- 1970 Günerken, E., D’Hondt, E., Eppink, M.H.M., Garcia-Gonzalez, L., Elst, K., Wijffels, R.H.,
 1971 2015b. Cell disruption for microalgae biorefineries. *Biotechnol. Adv.* 33, 243–260.
 1972 <https://doi.org/10.1016/j.biotechadv.2015.01.008>
- 1973 Günerken, E., D’Hondt, E., Eppink, M.H.M., Wijffels, R.H., Elst, K., 2019. Disruption of
 1974 microalgae with a novel continuous explosive decompression device. *Algal Res.* 39,
 1975 101376. <https://doi.org/10.1016/j.algal.2018.12.001>
- 1976 Günther, S., Gernat, D., Overbeck, A., Kampen, I., Kwade, A., 2016. Micromechanical
 1977 Properties and Energy Requirements of the Microalgae *Chlorella vulgaris* for Cell
 1978 Disruption. *Chem. Eng. Technol.* 39, 1693–1699.
 1979 <https://doi.org/10.1002/ceat.201400632>
- 1980 Hagen, C., Siegmund, S., Braune, W., 2002. Ultrastructural and chemical changes in the cell
 1981 wall of *Haematococcus pluvialis* (Volvocales, Chlorophyta) during aplanospore
 1982 formation. *Eur. J. Phycol.* 37, 217–226. <https://doi.org/10.1017/S0967026202003669>
- 1983 Halim, R., Harun, R., Danquah, M.K., Webley, P.A., 2012. Microalgal cell disruption for
 1984 biofuel development. *Appl. Energy* 91, 116–121.
 1985 <https://doi.org/10.1016/j.apenergy.2011.08.048>
- 1986 Halim, R., Rupasinghe, T.W.T., Tull, D.L., Webley, P.A., 2013. Mechanical cell disruption
 1987 for lipid extraction from microalgal biomass. *Bioresour. Technol.* 140, 53–63.
 1988 <https://doi.org/10.1016/j.biortech.2013.04.067>
- 1989 Halim, R., Webley, P.A., Martin, G.J.O., 2016. The CIDES process: Fractionation of
 1990 concentrated microalgal paste for co-production of biofuel, nutraceuticals, and high-
 1991 grade protein feed. *Algal Res.* 19, 299–306.
 1992 <https://doi.org/10.1016/j.algal.2015.09.018>
- 1993 Hannon, M., Gimpel, J., Tran, M., Rasala, B., Mayfield, S., 2010. Biofuels from algae:
 1994 challenges and potential. *Biofuels* 1, 763–784.
- 1995 Haque, F., Dutta, A., Thimmanagari, M., Chiang, Y.W., 2016. Intensified green production of
 1996 astaxanthin from *Haematococcus pluvialis*. *Food Bioprod. Process.* 99, 1–11.
 1997 <https://doi.org/10.1016/j.fbp.2016.03.002>

- 1998 Harith, Z.T., Yusoff, F.M., Mohamed, M.S., Shariff, M., Din, M.S.M., Ariff, A.B., 2009.
- 1999 Effect of different flocculants on the flocculation performance of microalgae,
- 2000 *Chaetoceros calcitrans*, cells. <https://doi.org/10.5897/ajb09.569>
- 2001 Hellebust, J.A., Ahmad, I., 1989. Regulation of Nitrogen Assimilation in Green Microalgae
- 2002 16.
- 2003 Hennart, S.L.A., Wildeboer, W.J., van Hee, P., Meesters, G.M.H., 2009. Identification of the
- 2004 grinding mechanisms and their origin in a stirred ball mill using population balances.
- 2005 *Chem. Eng. Sci.* 64, 4123–4130. <https://doi.org/10.1016/j.ces.2009.06.031>
- 2006 Heo, Y.M., Lee, H., Lee, C., Kang, J., Ahn, J.-W., Lee, Y.M., Kang, K.-Y., Choi, Y.-E., Kim,
- 2007 J.-J., 2017. An integrative process for obtaining lipids and glucose from *Chlorella*
- 2008 *vulgaris* biomass with a single treatment of cell disruption. *Algal Res.* 27, 286–294.
- 2009 <https://doi.org/10.1016/j.algal.2017.09.022>
- 2010 Hernández, D., Riaño, B., Coca, M., García-González, M.C., 2015. Saccharification of
- 2011 carbohydrates in microalgal biomass by physical, chemical and enzymatic pre-
- 2012 treatments as a previous step for bioethanol production. *Chem. Eng. J.* 262, 939–945.
- 2013 <https://doi.org/10.1016/j.cej.2014.10.049>
- 2014 Hom-Diaz, A., Passos, F., Ferrer, I., Vicent, T., Blánquez, P., 2016. Enzymatic pretreatment
- 2015 of microalgae using fungal broth from *Trametes versicolor* and commercial laccase for
- 2016 improved biogas production. *Algal Res.* 19, 184–188.
- 2017 <https://doi.org/10.1016/j.algal.2016.08.006>
- 2018 T.R. Hopkins, Physical and chemical cell disruption for the recovery of intracellular protein.
- 2019 Chapter 3, R. Seetharam, S. Sharma (Eds.), *Purification and analysis of recombinant proteins*,
- 2020 Macel Dekker, New York (1991), pp. 57-84
- 2021 Horvat, J., Klaić, B., Metelko, B., Šunjić, V., 1985. Mechanism of levulinic acid formation.
- 2022 *Tetrahedron Lett.* 26, 2111–2114. [https://doi.org/10.1016/S0040-4039\(00\)94793-2](https://doi.org/10.1016/S0040-4039(00)94793-2)
- 2023 Howlader, M.S., French, W.T., Shields-Menard, S.A., Amirsadeghi, M., Green, M., Rai, N.,
- 2024 2017. Microbial cell disruption for improving lipid recovery using pressurized CO₂:
- 2025 Role of CO₂ solubility in cell suspension, sugar broth, and spent media. *Biotechnol.*
- 2026 *Prog.* 33, 737–748. <https://doi.org/10.1002/btpr.2471>
- 2027 Hu, Y., Gong, M., Xu, C. (Charles), Bassi, A., 2017. Investigation of an alternative cell
- 2028 disruption approach for improving hydrothermal liquefaction of microalgae. *Fuel* 197,
- 2029 138–144. <https://doi.org/10.1016/j.fuel.2017.02.022>
- 2030 Huang, Y., Hong, A., Zhang, D., Li, L., 2014. Comparison of cell rupturing by ozonation and
- 2031 ultrasonication for algal lipid extraction from *Chlorella vulgaris*. *Environ. Technol.* 35,
- 2032 931–937. <https://doi.org/10.1080/09593330.2013.856954>
- 2033 Hung, M.T., Liu, J.C., 2018. Microfiltration of microalgae in the presence of rigid particles.
- 2034 *Sep. Purif. Technol., Filtering a Better Future* 198, 10–15.
- 2035 <https://doi.org/10.1016/j.seppur.2016.10.063>
- 2036 Jaeschke, D.P., Mercali, G.D., Marczak, L.D.F., Müller, G., Frey, W., Gusbeth, C., 2019.
- 2037 Extraction of valuable compounds from *Arthrospira platensis* using pulsed electric
- 2038 field treatment. *Bioresour. Technol.* 283, 207–212.
- 2039 <https://doi.org/10.1016/j.biortech.2019.03.035>
- 2040 Jana, A., Ghosh, S., Majumdar, S., 2018. Energy efficient harvesting of *Arthrospira* sp. using
- 2041 ceramic membranes: analyzing the effect of membrane pore size and incorporation of
- 2042 flocculant as fouling control strategy. *J. Chem. Technol. Biotechnol.* 93, 1085–1096.
- 2043 <https://doi.org/10.1002/jctb.5466>
- 2044 Jiang, S., Zhang, Y., Zhao, F., Yu, Z., Zhou, X., Chu, H., 2018. Impact of transmembrane
- 2045 pressure (TMP) on membrane fouling in microalgae harvesting with a uniform

2046 shearing vibration membrane system. *Algal Res.* 35, 613–623.
2047 <https://doi.org/10.1016/j.algal.2018.10.003>

2048 Johansen, J., 1991. Morphological Variability and Cell-Wall Composition of *Phaeodactylum-*
2049 *Tricornutum* (bacillariophyceae). *Gt. Basin Nat.* 51, 310–315.

2050 Juneja, A., Ceballos, R.M., Murthy, G.S., 2013. Effects of Environmental Factors and Nutrient
2051 Availability on the Biochemical Composition of Algae for Biofuels Production: A Review.
2052 *Energies* 6, 4607–4638. <https://doi.org/10.3390/en6094607>

2053 Kalina, T., Zabova, I., Hilgard, S., 1993. The Spectroscopy of the Acetoresistant Cell-Wall
2054 Biopolymers of *Auxenochlorella-Protothecoides*, *Scenedesmus-Quadricauda* and
2055 *Chlamydomonas-Geitleri* Zygospores (chlorophyta). *Arch. Hydrobiol.* 65–77.

2056 Kamaroddin, M.F., Hanotu, J., Gilmour, D.J., Zimmerman, W.B., 2016. In-situ disinfection
2057 and a new downstream processing scheme from algal harvesting to lipid extraction
2058 using ozone-rich microbubbles for biofuel production. *Algal Res.* 17, 217–226.
2059 <https://doi.org/10.1016/j.algal.2016.05.006>

2060 Kapaun, E., Reisser, W., 1995. A chitin-like glycan in the cell wall of a *Chlorella* sp.
2061 (*Chlorococcales*, *Chlorophyceae*). *Planta* 197, 577–582.

2062 Keris-Sen, U.D., Gurol, M.D., 2017. Using ozone for microalgal cell disruption to improve
2063 enzymatic saccharification of cellular carbohydrates. *Biomass Bioenergy* 105, 59–65.
2064 <https://doi.org/10.1016/j.biombioe.2017.06.023>

2065 Keris-Sen, U.D., Sen, U., Soydemir, G., Gurol, M.D., 2014. An investigation of ultrasound
2066 effect on microalgal cell integrity and lipid extraction efficiency. *Bioresour. Technol.*
2067 152, 407–413. <https://doi.org/10.1016/j.biortech.2013.11.018>

2068 Khairuddin, N.F.M., Idris, A., Hock, L.W., 2019. Harvesting *Nannochloropsis* sp. using
2069 PES/MWCNT/LiBr membrane with good antifouling properties. *Sep. Purif. Technol.*
2070 212, 1–11. <https://doi.org/10.1016/j.seppur.2018.11.013>

2071 Kim, D., Kwak, M., Kim, K., Chang, Y.K., 2019. Turbulent jet-assisted microfiltration for
2072 energy efficient harvesting of microalgae. *J. Membr. Sci.* 575, 170–178.
2073 <https://doi.org/10.1016/j.memsci.2018.12.069>

2074 Kim, G., Mujtaba, G., Lee, K., 2016. Effects of nitrogen sources on cell growth and biochemical
2075 composition of marine chlorophyte *Tetraselmis* sp. for lipid production. *ALGAE* 31,
2076 257–266. <https://doi.org/10.4490/algae.2016.31.8.18>

2077 Kim, J.K., Um, B.-H., Kim, T.H., 2012. Bioethanol production from micro-algae,
2078 *Schizocytrium* sp., using hydrothermal treatment and biological conversion. *Korean J.*
2079 *Chem. Eng.* 29, 209–214. <https://doi.org/10.1007/s11814-011-0169-3>

2080 Kim, S., Kim, Y.-S., 2018. *Chlorella* virus-mediated disruption of microalgal cell wall for
2081 biodiesel production. *Korean J. Microbiol.* 54, 140–145.
2082 <https://doi.org/10.7845/kjm.2018.8020>

2083 Krishna Koyande, A., Tanzil, V., Murrally Dharan, H., Subramaniam, M., Robert, R.N., Lau,
2084 P.-L., Khoiroh, I., Show, P.-L., 2020. Integration of osmotic shock assisted liquid
2085 biphasic system for protein extraction from microalgae *Chlorella vulgaris*. *Biochem.*
2086 *Eng. J.* 157, 107532. <https://doi.org/10.1016/j.bej.2020.107532>

2087 Kröger, M., Klemm, M., Nelles, M., 2018. Hydrothermal Disintegration and Extraction of
2088 Different Microalgae Species. *Energies* 11, 450. <https://doi.org/10.3390/en11020450>

2089 Krug, J.H.A., 2018. Accounting of GHG emissions and removals from forest management: a
2090 long road from Kyoto to Paris. *Carbon Balance Manag.* 13, 1.
2091 <https://doi.org/10.1186/s13021-017-0089-6>

2092 Kurokawa, M., King, P.M., Wu, X., Joyce, E.M., Mason, T.J., Yamamoto, K., 2016. Effect of
2093 sonication frequency on the disruption of algae. *Ultrason. Sonochem.* 31, 157–162.
2094 <https://doi.org/10.1016/j.ultsonch.2015.12.011>

2095 Kwade, A., 1999. Determination of the most important grinding mechanism in stirred media
2096 mills by calculating stress intensity and stress number. *Powder Technol.* 105, 382–
2097 388. [https://doi.org/10.1016/S0032-5910\(99\)00162-X](https://doi.org/10.1016/S0032-5910(99)00162-X)

2098 Laamanen, C.A., Scott, J.A., 2017. Development of heat-aided flocculation for flotation
2099 harvesting of microalgae. *Biomass Bioenergy* 107, 150–154.
2100 <https://doi.org/10.1016/j.biombioe.2017.09.020>

2101 Lam, G.P. 't, 2017. Harvesting and cell disruption of microalgae.
2102 <https://doi.org/10.18174/412263>

2103 Le Costaouëc, T., Unamunzaga, C., Mantecon, L., Helbert, W., 2017. New structural insights
2104 into the cell-wall polysaccharide of the diatom *Phaeodactylum tricornutum*. *Algal Res.*
2105 26, 172–179. <https://doi.org/10.1016/j.algal.2017.07.021>

2106 Lee, I., Han, J.-I., 2015a. Simultaneous treatment (cell disruption and lipid extraction) of wet
2107 microalgae using hydrodynamic cavitation for enhancing the lipid yield. *Bioresour.*
2108 *Technol.* 186, 246–251. <https://doi.org/10.1016/j.biortech.2015.03.045>

2109 Lee, I., Han, J.-I., 2015b. Hydrothermal-acid treatment for effectual extraction of
2110 eicosapentaenoic acid (EPA)-abundant lipids from *Nannochloropsis salina*. *Bioresour.*
2111 *Technol.* 191, 1–6. <https://doi.org/10.1016/j.biortech.2015.04.124>

2112 Lee, I., Park, J.-Y., Choi, S.-A., Oh, Y.-K., Han, J.-I., 2014. Hydrothermal nitric acid
2113 treatment for effectual lipid extraction from wet microalgae biomass. *Bioresour.*
2114 *Technol.* 172, 138–142. <https://doi.org/10.1016/j.biortech.2014.08.101>

2115 Lee, Soo Youn, Cho, J.M., Chang, Y.K., Oh, Y.-K., 2017. Cell disruption and lipid extraction
2116 for microalgal biorefineries: A review. *Bioresour. Technol., SI:Algal Biorefinery* 244,
2117 1317–1328. <https://doi.org/10.1016/j.biortech.2017.06.038>

2118 Lee, Sze Ying, Show, P.L., Ling, T.C., Chang, J.-S., 2017. Single-step disruption and protein
2119 recovery from *Chlorella vulgaris* using ultrasonication and ionic liquid buffer aqueous
2120 solutions as extractive solvents. *Biochem. Eng. J.* 124, 26–35.
2121 <https://doi.org/10.1016/j.bej.2017.04.009>

2122 Lee, Y.-K., 2001. Microalgal mass culture systems and methods: Their limitation and
2123 potential. *J. Appl. Phycol.* 13, 307–315. <https://doi.org/10.1023/A:1017560006941>

2124 Lei, X., Chen, Y., Shao, Z., Chen, Z., Li, Y., Zhu, H., Zhang, J., Zheng, W., Zheng, T., 2015.
2125 Effective harvesting of the microalgae *Chlorella vulgaris* via flocculation–flotation
2126 with bioflocculant. *Bioresour. Technol.* 198, 922–925.
2127 <https://doi.org/10.1016/j.biortech.2015.08.095>

2128 Leonhardt, L., Käferböck, A., Smetana, S., de Vos, R., Toepfl, S., Parniakov, O., 2020. Bio-
2129 refinery of *Chlorella sorokiniana* with pulsed electric field pre-treatment. *Bioresour.*
2130 *Technol.* 301, 122743. <https://doi.org/10.1016/j.biortech.2020.122743>

2131 Letelier-Gordo, C.O., Holdt, S.L., De Francisci, D., Karakashev, D.B., Angelidaki, I., 2014.
2132 Effective harvesting of the microalgae *Chlorella protothecoides* via bioflocculation
2133 with cationic starch. *Bioresour. Technol.* 167, 214–218.
2134 <https://doi.org/10.1016/j.biortech.2014.06.014>

2135 Lewandowski, I., Heinz, A., 2003. Delayed harvest of miscanthus—influences on biomass
2136 quantity and quality and environmental impacts of energy production. *Eur. J. Agron.*
2137 19, 45–63. [https://doi.org/10.1016/S1161-0301\(02\)00018-7](https://doi.org/10.1016/S1161-0301(02)00018-7)

2138 Lin, C.-C., Hong, P.K.A., 2013. A new processing scheme from algae suspension to collected
2139 lipid using sand filtration and ozonation. *Algal Res.* 2, 378–384.
2140 <https://doi.org/10.1016/j.algal.2013.06.001>

2141 Liu, C., Hao, Y., Jiang, J., Liu, W., 2017. Valorization of untreated rice bran towards
2142 bioflocculant using a lignocellulose-degrading strain and its use in microalgal biomass
2143 harvest. *Biotechnol. Biofuels* 10, 90. <https://doi.org/10.1186/s13068-017-0780-6>

2144 Liu, C.-H., Chang, C.-Y., Cheng, C.-L., Lee, D.-J., Chang, J.-S., 2012. Fermentative
2145 hydrogen production by *Clostridium butyricum* CGS5 using carbohydrate-rich
2146 microalgal biomass as feedstock. *Int. J. Hydrog. Energy*, The 2011 Asian Bio-
2147 Hydrogen and Biorefinery Symposium (2011ABBS) 37, 15458–15464.
2148 <https://doi.org/10.1016/j.ijhydene.2012.04.076>

2149 Liu, Z.-W., Zeng, X.-A., Cheng, J.-H., Liu, D.-B., Aadil, R.M., 2018. The efficiency and
2150 comparison of novel techniques for cell wall disruption in astaxanthin extraction from
2151 *Haematococcus pluvialis*. *Int. J. Food Sci. Technol.* 53, 2212–2219.
2152 <https://doi.org/10.1111/ijfs.13810>

2153 Liu, S., Liu, C., Huang, X., Chai, Y., Cong, B., 2006. Optimization of Parameters for
2154 Isolation of Protoplasts from the Antarctic Sea Ice Alga *Chlamydomonas* Sp. ICE-L. *J Appl*
2155 *Phycol* 18, 783. <https://doi.org/10.1007/s10811-006-9093-z>

2156 Lorente, E., Farriol, X., Salvadó, J., 2015. Steam explosion as a fractionation step in biofuel
2157 production from microalgae. *Fuel Process. Technol.* 131, 93–98.
2158 <https://doi.org/10.1016/j.fuproc.2014.11.009>

2159 Lorente, E., Hapońska, M., Clavero, E., Torras, C., Salvadó, J., 2018. Steam Explosion and
2160 Vibrating Membrane Filtration to Improve the Processing Cost of Microalgae Cell
2161 Disruption and Fractionation. *Processes* 6, 28. <https://doi.org/10.3390/pr6040028>

2162 Lorente, E., Hapońska, M., Clavero, E., Torras, C., Salvadó, J., 2017. Microalgae
2163 fractionation using steam explosion, dynamic and tangential cross-flow membrane
2164 filtration. *Bioresour. Technol.*, 1st International Conference on Bioresource
2165 Technology for Bioenergy, Bioproducts & Environmental Sustainability
2166 (BIORESTEC) 237, 3–10. <https://doi.org/10.1016/j.biortech.2017.03.129>

2167 Luengo, E., Martínez, J.M., Bordetas, A., Álvarez, I., Raso, J., 2015. Influence of the
2168 treatment medium temperature on lutein extraction assisted by pulsed electric fields
2169 from *Chlorella vulgaris*. *Innov. Food Sci. Emerg. Technol.*, APPLICATIONS OF PEF
2170 FOR FOOD PROCESSING 29, 15–22. <https://doi.org/10.1016/j.ifset.2015.02.012>

2171 Maga, D., 2017. Life cycle assessment of biomethane produced from microalgae grown in
2172 municipal waste water. *Biomass Convers. Biorefinery* 7, 1–10.
2173 <https://doi.org/10.1007/s13399-016-0208-8>

2174 Mahdy, A., Mendez, L., Ballesteros, M., González-Fernández, C., 2014a. Autohydrolysis and
2175 alkaline pretreatment effect on *Chlorella vulgaris* and *Scenedesmus* sp. methane
2176 production. *Energy* 78, 48–52. <https://doi.org/10.1016/j.energy.2014.05.052>

2177 Mahdy, A., Mendez, L., Ballesteros, M., González-Fernández, C., 2014b. Enhanced methane
2178 production of *Chlorella vulgaris* and *Chlamydomonas reinhardtii* by hydrolytic
2179 enzymes addition. *Energy Convers. Manag.* 85, 551–557.
2180 <https://doi.org/10.1016/j.enconman.2014.04.097>

2181 Martín Juárez, J., Riol Pastor, E., Fernández Sevilla, J.M., Muñoz Torre, R., García-Encina,
2182 P.A., Bolado Rodríguez, S., 2018. Effect of pretreatments on biogas production from
2183 microalgae biomass grown in pig manure treatment plants. *Bioresour. Technol.* 257,
2184 30–38. <https://doi.org/10.1016/j.biortech.2018.02.063>

2185 Martínez, J.M., Gojkovic, Z., Ferro, L., Maza, M., Álvarez, I., Raso, J., Funk, C., 2019. Use
2186 of pulsed electric field permeabilization to extract astaxanthin from the Nordic
2187 microalga *Haematococcus pluvialis*. *Bioresour. Technol.* 289, 121694.
2188 <https://doi.org/10.1016/j.biortech.2019.121694>

2189 Masojídék, J., Torzillo, G., Koblížek, M., 2013. Photosynthesis in Microalgae, in: *Handbook*
2190 *of Microalgal Culture*. John Wiley & Sons, Ltd, pp. 21–36.
2191 <https://doi.org/10.1002/9781118567166.ch2>

- 2192 Mata, T.M., Mendes, A.M., Caetano, N.S., Martins, A.A., 2014. Sustainability and economic
2193 evaluation of microalgae grown in brewery wastewater. *Bioresour. Technol.*, Special
2194 Issue on Advance Biological Treatment Technologies for Sustainable Waste
2195 Management (ICSWHK2013) 168, 151–158.
2196 <https://doi.org/10.1016/j.biortech.2014.04.091>
- 2197 Mathimani, T., Mallick, N., 2018. A comprehensive review on harvesting of microalgae for
2198 biodiesel – Key challenges and future directions. *Renew. Sustain. Energy Rev.* 91,
2199 1103–1120. <https://doi.org/10.1016/j.rser.2018.04.083>
- 2200 Matos, Â.P., Teixeira, M.S., Corrêa, F.M.P.S., Machado, M.M., Werner, R.I.S., Aguiar, A.C.,
2201 Cubas, A.L.V., Sant'Anna, E.S., Moecke, E.H.S., 2019. DISRUPTION OF
2202 *Nannochloropsis gaditana* (EUSTIGMATOPHYCEAE) RIGID CELL WALL BY
2203 NON-THERMAL PLASMA PRIOR TO LIPID EXTRACTION AND ITS EFFECT
2204 ON FATTY ACID COMPOSITION. *Braz. J. Chem. Eng.* 36, 1419–1428.
2205 <https://doi.org/10.1590/0104-6632.20190364s20190097>
- 2206 Matter, I.A., Darwesh, O.M., Eida, M.F., 2018. Harvesting of microalgae *Scenedesmus*
2207 *obliquus* using chitosan-alginate dual flocculation system.
- 2208 McMillan, J.R., Watson, I.A., Ali, M., Jaafar, W., 2013. Evaluation and comparison of algal
2209 cell disruption methods: Microwave, waterbath, blender, ultrasonic and laser
2210 treatment. *Appl. Energy* 103, 128–134.
2211 <https://doi.org/10.1016/j.apenergy.2012.09.020>
- 2212 Mendes-Pinto, M.M., Raposo, M.F.J., Bowen, J., Young, A.J., Morais, R., 2001. Evaluation
2213 of different cell disruption processes on encysted cells of *Haematococcus pluvialis*:
2214 effects on astaxanthin recovery and implications for bio-availability. *J. Appl. Phycol.*
2215 13, 19–24. <https://doi.org/10.1023/A:1008183429747>
- 2216 Mendez, L., Mahdy, A., Timmers, R.A., Ballesteros, M., González-Fernández, C., 2013.
2217 Enhancing methane production of *Chlorella vulgaris* via thermochemical
2218 pretreatments. *Bioresour. Technol.* 149, 136–141.
2219 <https://doi.org/10.1016/j.biortech.2013.08.136>
- 2220 Milledge, J.J., Heaven, S., 2017. Energy Balance of Biogas Production from Microalgae:
2221 Effect of Harvesting Method, Multiple Raceways, Scale of Plant and Combined Heat
2222 and Power Generation. *J. Mar. Sci. Eng.* 5, 9. <https://doi.org/10.3390/jmse5010009>
- 2223 Milledge, J.J., Heaven, S., 2011. Disc stack centrifugation separation and cell disruption of
2224 microalgae: A technical note. *Environ. Nat. Resour. Res.* 1, 17–24.
2225 <https://doi.org/10.5539/enrr.v1n1p17>
- 2226 Molazadeh, M., Ahmadzadeh, H., Pourianfar, H.R., Lyon, S., Rampelotto, P.H., 2019. The
2227 Use of Microalgae for Coupling Wastewater Treatment With CO₂ Biofixation. *Front.*
2228 *Bioeng. Biotechnol.* 7. <https://doi.org/10.3389/fbioe.2019.00042>
- 2229 Montalescot, V., Rinaldi, T., Touchard, R., Jubeau, S., Frappart, M., Jaouen, P., Bourseau, P.,
2230 Marchal, L., 2015. Optimization of bead milling parameters for the cell disruption of
2231 microalgae: Process modeling and application to *Porphyridium cruentum* and
2232 *Nannochloropsis oculata*. *Bioresour. Technol.* 196, 339–346.
2233 <https://doi.org/10.1016/j.biortech.2015.07.075>
- 2234 Montsant, A., Zarka, A., Boussiba, S., 2001. Presence of a Nonhydrolyzable Biopolymer in
2235 the Cell Wall of Vegetative Cells and Astaxanthin-Rich Cysts of *Haematococcus*
2236 *pluvialis* (Chlorophyceae). *Mar. Biotechnol.* 3, 515–521.
2237 <https://doi.org/10.1007/s1012601-0051-0>
- 2238 Mussatto, S.I., Roberto, I.C., 2004. Alternatives for detoxification of diluted-acid
2239 lignocellulosic hydrolyzates for use in fermentative processes: a review. *Bioresour.*
2240 *Technol.* 93, 1–10. <https://doi.org/10.1016/j.biortech.2003.10.005>

2241 Muylaert, K., Bastiaens, L., Vandamme, D., Gouveia, L., 2017. 5 - Harvesting of microalgae:
2242 Overview of process options and their strengths and drawbacks, in: Gonzalez-
2243 Fernandez, C., Muñoz, R. (Eds.), *Microalgae-Based Biofuels and Bioproducts*,
2244 Woodhead Publishing Series in Energy. Woodhead Publishing, pp. 113–132.
2245 <https://doi.org/10.1016/B978-0-08-101023-5.00005-4>

2246 Nascimento, I.A., Marques, S.S.I., Cabanelas, I.T.D., de Carvalho, G.C., Nascimento, M.A.,
2247 de Souza, C.O., Druzian, J.I., Hussain, J., Liao, W., 2014. Microalgae Versus Land
2248 Crops as Feedstock for Biodiesel: Productivity, Quality, and Standard Compliance.
2249 *BioEnergy Res.* 7, 1002–1013. <https://doi.org/10.1007/s12155-014-9440-x>

2250 Nava Bravo, I., Velásquez-Orta, S.B., Cuevas-García, R., Monje-Ramírez, I., Harvey, A.,
2251 Orta Ledesma, M.T., 2019. Bio-crude oil production using catalytic hydrothermal
2252 liquefaction (HTL) from native microalgae harvested by ozone-flotation. *Fuel* 241,
2253 255–263. <https://doi.org/10.1016/j.fuel.2018.12.071>

2254 Neilson, A.H., Lewin, R.A., 1974. The uptake and utilization of organic carbon by algae: an
2255 essay in comparative biochemistry. *Phycologia* 13, 227–264.
2256 <https://doi.org/10.2216/i0031-8884-13-3-227.1>

2257 Němcová, Y., Kalina, T., 2000. Cell wall development, microfibril and pyrenoid structure in
2258 type strains of *Chlorella vulgaris*, *C. kessleri*, *C. sorokiniana* compared with *C. luteoviridis*
2259 (*Trebouxiophyceae*, *Chlorophyta*). *archiv_algolstud* 100, 95–105.
2260 https://doi.org/10.1127/algol_stud/100/2000/95

2261 Ngamsirisomsakul, M., Reungsang, A., Liao, Q., Kongkeitkajorn, M.B., 2019. Enhanced bio-
2262 ethanol production from *Chlorella* sp. biomass by hydrothermal pretreatment and
2263 enzymatic hydrolysis. *Renew. Energy* 141, 482–492.
2264 <https://doi.org/10.1016/j.renene.2019.04.008>

2265 Niaghi, M., Mahdavi, M.A., Gheshlaghi, R., 2015. Optimization of dissolved air flotation
2266 technique in harvesting microalgae from treated wastewater without flocculants
2267 addition. *J. Renew. Sustain. Energy* 7, 013130. <https://doi.org/10.1063/1.4909541>

2268 Nitsos, C.K., Mihailof, C.M., Matis, K.A., Lappas, A.A., Triantafyllidis, K.S., 2013. Chapter
2269 7 - The Role of Catalytic Pretreatment in Biomass Valorization Toward Fuels and
2270 Chemicals, in: Triantafyllidis, K.S., Lappas, A.A., Stöcker, M. (Eds.), *The Role of
2271 Catalysis for the Sustainable Production of Bio-Fuels and Bio-Chemicals*. Elsevier,
2272 Amsterdam, pp. 217–260. <https://doi.org/10.1016/B978-0-444-56330-9.00007-3>

2273 Nitsos, C.K., Matis, K.A., Triantafyllidis, K.S., 2013. Optimization of Hydrothermal
2274 Pretreatment of Lignocellulosic Biomass in the Bioethanol Production Process.
2275 *ChemSusChem* 6, 110–122. <https://doi.org/10.1002/cssc.201200546>

2276 Obeid, S., Beaufils, N., Camy, S., Takache, H., Ismail, A., Pontalier, P.-Y., 2018.
2277 Supercritical carbon dioxide extraction and fractionation of lipids from freeze-dried
2278 microalgae *Nannochloropsis oculata* and *Chlorella vulgaris*. *Algal Res.* 34, 49–56.
2279 <https://doi.org/10.1016/j.algal.2018.07.003>

2280 Olmstead, I.L.D., Kentish, S.E., Scales, P.J., Martin, G.J.O., 2013. Low solvent, low
2281 temperature method for extracting biodiesel lipids from concentrated microalgal
2282 biomass. *Bioresour. Technol.* 148, 615–619.
2283 <https://doi.org/10.1016/j.biortech.2013.09.022>

2284 Onay, M., 2019. Bioethanol production via different saccharification strategies from *H.*
2285 *tetrachotoma* ME03 grown at various concentrations of municipal wastewater in a flat-
2286 photobioreactor. *Fuel* 239, 1315–1323. <https://doi.org/10.1016/j.fuel.2018.11.126>

2287 Onumaegbu, C., Alaswad, A., Rodriguez, C., Olabi, A.G., 2018. Optimization of Pre-
2288 Treatment Process Parameters to Generate Biodiesel from Microalga. *Energies* 11,
2289 806. <https://doi.org/10.3390/en11040806>

- 2290 Orlova, T.Yu., Sabutskaya, M.A., Markina, Zh.V., 2019. Ultrastructural Changes in Marine
2291 Microalgae from Different Taxonomic Groups during Batch Cultivation. *Russ. J. Mar.*
2292 *Biol.* 45, 202–210. <https://doi.org/10.1134/S1063074019030106>
- 2293 Orr, V.C.A., Plechkova, N.V., Seddon, K.R., Rehmann, L., 2016. Disruption and Wet
2294 Extraction of the Microalgae *Chlorella vulgaris* Using Room-Temperature Ionic
2295 Liquids. *ACS Sustain. Chem. Eng.* 4, 591–600.
2296 <https://doi.org/10.1021/acssuschemeng.5b00967>
- 2297 Palmqvist, E., Hahn-Hägerdal, B., 2000. Fermentation of lignocellulosic hydrolysates. I:
2298 inhibition and detoxification. *Bioresour. Technol.* 74, 17–24.
2299 [https://doi.org/10.1016/S0960-8524\(99\)00160-1](https://doi.org/10.1016/S0960-8524(99)00160-1)
- 2300 Pan, Z., Huang, Y., Wang, Y., Wu, Z., 2017. Disintegration of *Nannochloropsis* sp. cells in an
2301 improved turbine bead mill. *Bioresour. Technol.* 245, 641–648.
2302 <https://doi.org/10.1016/j.biortech.2017.08.146>
- 2303 Paredes-López, O., Camargo-Rubio, E., Ornelas-Vale, A., 1976. Influence of specific growth
2304 rate on biomass yield, productivity, and composition of *Candida utilis* in batch and
2305 continuous culture. *Appl. Environ. Microbiol.* 31, 487–491.
- 2306 Park, J.B.K., Craggs, R.J., Shilton, A.N., 2011. Recycling algae to improve species control
2307 and harvest efficiency from a high rate algal pond. *Water Res.* 45, 6637–6649.
2308 <https://doi.org/10.1016/j.watres.2011.09.042>
- 2309 Passos, F., Carretero, J., Ferrer, I., 2015. Comparing pretreatment methods for improving
2310 microalgae anaerobic digestion: Thermal, hydrothermal, microwave and ultrasound.
2311 *Chem. Eng. J.* 279, 667–672. <https://doi.org/10.1016/j.cej.2015.05.065>
- 2312 Peperzak, L., 2003. Phytoplankton sinking rates in the Rhine region of freshwater influence.
2313 *Journal of Plankton Research* 25, 365–383. <https://doi.org/10.1093/plankt/25.4.365>
- 2314 Phong, W.N., Le, C.F., Show, P.L., Chang, J.-S., Ling, T.C., 2017. Extractive disruption
2315 process integration using ultrasonication and an aqueous two-phase system for protein
2316 recovery from *Chlorella sorokiniana*. *Eng. Life Sci.* 17, 357–369.
2317 <https://doi.org/10.1002/elsc.201600133>
- 2318 Phong, W.N., Show, P.L., Le, C.F., Tao, Y., Chang, J.-S., Ling, T.C., 2018. Improving cell
2319 disruption efficiency to facilitate protein release from microalgae using chemical and
2320 mechanical integrated method. *Biochem. Eng. J.* 135, 83–90.
2321 <https://doi.org/10.1016/j.bej.2018.04.002>
- 2322 Picart, L., Cheftel, J.-C., 2003. 18 - Pulsed electric fields, in: Zeuthen, P., Bøgh-Sørensen, L.
2323 (Eds.), *Food Preservation Techniques*, Woodhead Publishing Series in Food Science,
2324 *Technology and Nutrition*. Woodhead Publishing, pp. 360–427.
2325 <https://doi.org/10.1533/9781855737143.3.360>
- 2326 Polat, E., Yüksel, E. & Altınbaş, M., 2020. Mutual effect of sodium and magnesium on the
2327 cultivation of microalgae *Auxenochlorella protothecoides*. *Biomass and Bioenergy*
2328 132, 105441.
- 2329 Postma, P.R., Suarez-Garcia, E., Safi, C., Yonathan, K., Olivieri, G., Barbosa, M.J., Wijffels,
2330 R.H., Eppink, M.H.M., 2017. Energy efficient bead milling of microalgae: Effect of
2331 bead size on disintegration and release of proteins and carbohydrates. *Bioresour.*
2332 *Technol.* 224, 670–679. <https://doi.org/10.1016/j.biortech.2016.11.071>
- 2333 Qu, Z., Zeng, J., Zhang, Y., Liao, Q., Sharma, B.K., Fu, Q., Huang, Y., Liu, Z., 2018.
2334 Hydrothermal cell disruption of *Nannochloropsis* sp. and its influence on lipid
2335 extraction. *Algal Res.* 35, 407–415. <https://doi.org/10.1016/j.algal.2018.09.015>
- 2336 Raja, R., Coelho, A., Hemaiswarya, S., Kumar, P., Carvalho, I.S., Alagarsamy, A., 2018.
2337 Applications of microalgal paste and powder as food and feed: An update using text
2338 mining tool. *Beni-Suef Univ. J. Basic Appl. Sci.* 7, 740–747.
2339 <https://doi.org/10.1016/j.bjbas.2018.10.004>

- 2340 Ramos Tercero, E.A., Domenicali, G., Bertucco, A., 2014. Autotrophic production of
 2341 biodiesel from microalgae: An updated process and economic analysis. *Energy* 76,
 2342 807–815. <https://doi.org/10.1016/j.energy.2014.08.077>
- 2343 Remias, D., Karsten, U., Lütz, C., Leya, T., 2010. Physiological and morphological processes
 2344 in the Alpine snow alga *Chloromonas nivalis* (Chlorophyceae) during cyst formation.
 2345 *Protoplasma* 243, 73–86. <https://doi.org/10.1007/s00709-010-0123-y>
- 2346 Ras, M., Steyer, J.-P., Bernard, O., 2013. Temperature effect on microalgae: a crucial factor
 2347 for outdoor production. *Rev. Environ. Sci. Biotechnol.* 12, 153–164.
 2348 <https://doi.org/10.1007/s11157-013-9310-6>
- 2349 Razzak, S.A., Hossain, M.M., Lucky, R.A., Bassi, A.S., de Lasa, H., 2013. Integrated CO2
 2350 capture, wastewater treatment and biofuel production by microalgae culturing—A
 2351 review. *Renew. Sustain. Energy Rev.* 27, 622–653.
 2352 <https://doi.org/10.1016/j.rser.2013.05.063>
- 2353 Reyes, F.A., Sielfeld, C.S., del Valle, J.M., 2016. Effect of high-pressure compaction on
 2354 supercritical CO2 extraction of astaxanthin from *Haematococcus pluvialis*. *J. Food*
 2355 *Eng.* 189, 123–134. <https://doi.org/10.1016/j.jfoodeng.2016.05.008>
- 2356 Rodrigues, M.A., da Silva Bon, E.P., 2011. Evaluation of *Chlorella* (Chlorophyta) as Source
 2357 of Fermentable Sugars via Cell Wall Enzymatic Hydrolysis. *Enzyme Res.* 2011.
 2358 <https://doi.org/10.4061/2011/405603>
- 2359 Rodríguez-Meizoso, I., Jaime, L., Santoyo, S., Señoráns, F.J., Cifuentes, A., Ibáñez, E., 2010.
 2360 Subcritical water extraction and characterization of bioactive compounds from
 2361 *Haematococcus pluvialis* microalga. *J. Pharm. Biomed. Anal., Natural Bioactive*
 2362 *Compounds and Nutrigenomics* 51, 456–463.
 2363 <https://doi.org/10.1016/j.jpba.2009.03.014>
- 2364 Rounsaville, J., Rice, R.G., 1996. Evolution of ozone for the bleaching of paper pulps. *Ozone*
 2365 *Sci. Eng.* 18, 549–566. <https://doi.org/10.1080/01919512.1997.10382863>
- 2366 Ruiz, H.A., Silva, D.P., Ruzene, D.S., Lima, L.F., Vicente, A.A., Teixeira, J.A., 2012.
 2367 Bioethanol production from hydrothermal pretreated wheat straw by a flocculating
 2368 *Saccharomyces cerevisiae* strain – Effect of process conditions. *Fuel* 95, 528–536.
 2369 <https://doi.org/10.1016/j.fuel.2011.10.060>
- 2370 Ruiz-Ruiz, F., Benavides, J., Rito-Palomares, M., 2013. Scaling-up of a B-phycoerythrin
 2371 production and purification bioprocess involving aqueous two-phase systems:
 2372 Practical experiences. *Process Biochem.* 48, 738–745.
 2373 <https://doi.org/10.1016/j.procbio.2013.02.010>
- 2374 Ryckebosch, E., Muylaert, K., Eeckhout, M., Ruysen, T., Foubert, I., 2011. Influence of
 2375 drying and storage on lipid and carotenoid stability of the microalga *Phaeodactylum*
 2376 *tricornutum*. *J. Agric. Food Chem.* 59, 11063–11069.
 2377 <https://doi.org/10.1021/jf2025456>
- 2378 Safi, C., Camy, S., Frances, C., Varela, M.M., Badia, E.C., Pontalier, P.-Y., Vaca-Garcia, C.,
 2379 2014. Extraction of lipids and pigments of *Chlorella vulgaris* by supercritical carbon
 2380 dioxide: influence of bead milling on extraction performance. *J. Appl. Phycol.* 26,
 2381 1711–1718. <https://doi.org/10.1007/s10811-013-0212-3>
- 2382 Safi, C., Cabas Rodriguez, L., Mulder, W.J., Engelen-Smit, N., Spekking, W., van den Broek,
 2383 L.A.M., Olivieri, G., Sijtsma, L., 2017a. Energy consumption and water-soluble
 2384 protein release by cell wall disruption of *Nannochloropsis gaditana*. *Bioresour.*
 2385 *Technol.* 239, 204–210. <https://doi.org/10.1016/j.biortech.2017.05.012>
- 2386 Safi, C., Olivieri, G., Campos, R.P., Engelen-Smit, N., Mulder, W.J., van den Broek, L.A.M.,
 2387 Sijtsma, L., 2017b. Biorefinery of microalgal soluble proteins by sequential processing
 2388 and membrane filtration. *Bioresour. Technol.* 225, 151–158.
 2389 <https://doi.org/10.1016/j.biortech.2016.11.068>

- 2390 Sahoo, N.K., Gupta, S.K., Rawat, I., Ansari, F.A., Singh, P., Naik, S.N., Bux, F., 2017.
 2391 Sustainable dewatering and drying of self-flocculating microalgae and study of cake
 2392 properties. *J. Clean. Prod.* 159, 248–256. <https://doi.org/10.1016/j.jclepro.2017.05.015>
 2393 Samarasinghe, N., Fernando, S., Lacey, R., Faulkner, W.B., 2012. Algal cell rupture using
 2394 high pressure homogenization as a prelude to oil extraction. *Renew. Energy* 48, 300–
 2395 308. <https://doi.org/10.1016/j.renene.2012.04.039>
 2396 Shankar, M., Chhotaray, P.K., Agrawal, A., Gardas, R.L., Tamilarasan, K., Rajesh, M., 2017.
 2397 Protic ionic liquid-assisted cell disruption and lipid extraction from fresh water
 2398 *Chlorella* and *Chlorococcum* microalgae. *Algal Res.* 25, 228–236.
 2399 <https://doi.org/10.1016/j.algal.2017.05.009>
 2400 Scherer, D., Krust, D., Frey, W., Mueller, G., Nick, P., Gusbeth, C., 2019. Pulsed electric
 2401 field (PEF)-assisted protein recovery from *Chlorella vulgaris* is mediated by an
 2402 enzymatic process after cell death. *Algal Res.* 41, 101536.
 2403 <https://doi.org/10.1016/j.algal.2019.101536>
 2404 Seo, Y.H., Sung, M., Oh, Y.-K., Han, J.-I., 2016. Lipid extraction from microalgae cell using
 2405 persulfate-based oxidation. *Bioresour. Technol.* 200, 1073–1075.
 2406 <https://doi.org/10.1016/j.biortech.2015.10.106>
 2407 Seo, Y.H., Sung, M., Oh, Y.-K., Han, J.-I., 2015a. Lipid extraction from microalgae cell using
 2408 UV–Fenton-like reaction. *Bioresour. Technol.* 192, 792–794.
 2409 <https://doi.org/10.1016/j.biortech.2015.04.108>
 2410 Seo, Y.H., Sung, M., Oh, Y.-K., Han, J.-I., 2015b. Lipid extraction and esterification for
 2411 microalgae-based biodiesel production using pyrite (FeS₂). *Bioresour. Technol.* 191,
 2412 420–425. <https://doi.org/10.1016/j.biortech.2015.02.083>
 2413 Shannon, E., Abu-Ghannam, N., 2018. Enzymatic extraction of fucoxanthin from brown
 2414 seaweeds. *Int. J. Food Sci. Technol.* 53, 2195–2204. <https://doi.org/10.1111/ijfs.13808>
 2415 Shelef, G., Sukenik, A., Green, M., 1984. Microalgae harvesting and processing: a literature
 2416 review (No. SERI/STR-231-2396, 6204677). <https://doi.org/10.2172/6204677>
 2417 Shene, C., Monsalve, M.T., Vergara, D., Lienqueo, M.E., Rubilar, M., 2016. High pressure
 2418 homogenization of *Nannochloropsis oculata* for the extraction of intracellular
 2419 components: Effect of process conditions and culture age. *Eur. J. Lipid Sci. Technol.*
 2420 118, 631–639. <https://doi.org/10.1002/ejlt.201500011>
 2421 Sheng, J., Vannela, R., Rittmann, B.E., 2011. Evaluation of Cell-Disruption Effects of Pulsed-
 2422 Electric-Field Treatment of *Synechocystis* PCC 6803. *Environ. Sci. Technol.* 45,
 2423 3795–3802. <https://doi.org/10.1021/es103339x>
 2424 Shi, W., Zhu, L., Chen, Q., Lu, J., Pan, G., Hu, L., Yi, Q., 2017. Synergy of flocculation and
 2425 flotation for microalgae harvesting using aluminium electrolysis. *Bioresour. Technol.*
 2426 233, 127–133. <https://doi.org/10.1016/j.biortech.2017.02.084>
 2427 Shokrkar, H., Ebrahimi, S., Zamani, M., 2017. Bioethanol production from acidic and
 2428 enzymatic hydrolysates of mixed microalgae culture. *Fuel* 200, 380–386.
 2429 <https://doi.org/10.1016/j.fuel.2017.03.090>
 2430 Sierra, L.S., Dixon, C.K., Wilken, L.R., 2017. Enzymatic cell disruption of the microalgae
 2431 *Chlamydomonas reinhardtii* for lipid and protein extraction. *Algal Res.* 25, 149–159.
 2432 <https://doi.org/10.1016/j.algal.2017.04.004>
 2433 Simpson, R.J., 2010. Disruption of Cultured Cells by Nitrogen Cavitation. Cold Spring Harb.
 2434 *Protoc.* 2010, pdb.prot5513. <https://doi.org/10.1101/pdb.prot5513>
 2435 Singh, D., Nedbal, L., Ebenhöh, O., 2018. Modelling phosphorus uptake in microalgae.
 2436 *Biochem. Soc. Trans.* 46, 483–490. <https://doi.org/10.1042/BST20170262>
 2437 Şirin, S., Clavero, E., Salvadó, J., 2015. Efficient harvesting of *Chaetoceros calcitrans* for
 2438 biodiesel production. *Environ. Technol.* 36, 1902–1912.
 2439 <https://doi.org/10.1080/09593330.2015.1015456>

- 2440 Solé-Bundó, M., Carrère, H., Garfí, M., Ferrer, I., 2017. Enhancement of microalgae
 2441 anaerobic digestion by thermo-alkaline pretreatment with lime (CaO). *Algal Res.* 24,
 2442 199–206. <https://doi.org/10.1016/j.algal.2017.03.025>
- 2443 Solovchenko, A.E., Ismagulova, T.T., Lukyanov, A.A., Vasilieva, S.G., Konyukhov, I.V.,
 2444 Pogosyan, S.I., Lobakova, E.S., Gorelova, O.A., 2019. Luxury phosphorus uptake in
 2445 microalgae. *J. Appl. Phycol.* 31, 2755–2770. <https://doi.org/10.1007/s10811-019-01831-8>
- 2447 Spiden, E.M., Yap, B.H.J., Hill, D.R.A., Kentish, S.E., Scales, P.J., Martin, G.J.O., 2013.
 2448 Quantitative evaluation of the ease of rupture of industrially promising microalgae by
 2449 high pressure homogenization. *Bioresour. Technol.* 140, 165–171.
 2450 <https://doi.org/10.1016/j.biortech.2013.04.074>
- 2451 Steriti, A., Rossi, R., Concas, A., Cao, G., 2014. A novel cell disruption technique to enhance
 2452 lipid extraction from microalgae. *Bioresour. Technol.* 164, 70–77.
 2453 <https://doi.org/10.1016/j.biortech.2014.04.056>
- 2454 Sumprasit, N., Wagle, N., Glanpracha, N., Annachhatre, A.P., 2017. Biodiesel and biogas
 2455 recovery from *Spirulina platensis*. *Int. Biodeterior. Biodegrad., Environmental*
 2456 *Biotechnologies for Sustainable Development (EBSuD)* 119, 196–204.
 2457 <https://doi.org/10.1016/j.ibiod.2016.11.006>
- 2458 T Lam, G.P., Van Der Kolk, J.A., Chordia, A., Vermuë, M.H., Olivieri, G., Eppink, M.H.M.,
 2459 Wijffels, R.H., 2017. Mild and Selective Protein Release of Cell Wall Deficient
 2460 Microalgae with Pulsed Electric Field. *ACS Sustain. Chem. Eng.* 5, 6046–6053.
 2461 <https://doi.org/10.1021/acssuschemeng.7b00892>
- 2462 Taghavijeloudar, M., Park, J., Hashemi, S., Han, M., 2019. The effects of surfactants (sodium
 2463 dodecyl sulfate, triton X-100 and cetyl trimethyl ammonium bromide) on the
 2464 dewaterability of microalgae biomass using pressure filtration. *Bioresour. Technol.*
 2465 273, 565–572. <https://doi.org/10.1016/j.biortech.2018.11.062>
- 2466 Taher, H., Al-Zuhair, S., Al-Marzouqi, A.H., Haik, Y., Farid, M., 2014. Effective extraction
 2467 of microalgae lipids from wet biomass for biodiesel production. *Biomass Bioenergy*
 2468 66, 159–167. <https://doi.org/10.1016/j.biombioe.2014.02.034>
- 2469 Teixeira, R.E., 2012. Energy-efficient extraction of fuel and chemical feedstocks from algae.
 2470 *Green Chem.* 14, 419–427. <https://doi.org/10.1039/C2GC16225C>
- 2471 Throndsen, J., 1997. Chapter 5 - The Planktonic Marine Flagellates, in: Tomas, C.R. (Ed.),
 2472 *Identifying Marine Phytoplankton*. Academic Press, San Diego, pp. 591–729.
 2473 <https://doi.org/10.1016/B978-012693018-4/50007-0>
- 2474 Thu, N.T.H., Anh, H.T.L., Hoang, M.H., Kim, D.D., Hong, D.D., 2015. Study on biological
 2475 characteristics of a newly isolated Vietnamese strain of microalga *Isochrysis galbana*
 2476 Parke for utilizing as live aquaculture feed. *Russ. J. Mar. Biol.* 41, 203–211.
 2477 <https://doi.org/10.1134/S1063074015030074>
- 2478 Tilton, R.C., Murphy, J., Dixon, J.K., 1972. The flocculation of algae with synthetic
 2479 polymeric flocculants. *Water Res.* 6, 155–164. [https://doi.org/10.1016/0043-1354\(72\)90090-5](https://doi.org/10.1016/0043-1354(72)90090-5)
- 2481 Tsutsumi, S., Yokomizo, M., Saito, Y., Matsushita, Y., Aoki, H., 2017. Mechanical cell
 2482 disruption of microalgae for investigating the effects of degree of disruption on
 2483 hydrocarbon extraction. *Asia-Pac. J. Chem. Eng.* 12, 454–467.
 2484 <https://doi.org/10.1002/apj.2088>
- 2485 Udom, I., Zaribaf, B.H., Halfhide, T., Gillie, B., Dalrymple, O., Zhang, Q., Ergas, S.J., 2013.
 2486 Harvesting microalgae grown on wastewater. *Bioresour. Technol.* 139, 101–106.
 2487 <https://doi.org/10.1016/j.biortech.2013.04.002>

- 2488 Uduman, N., Qi, Y., Danquah, M.K., Hoadley, A.F.A., 2010. Marine microalgae flocculation
2489 and focused beam reflectance measurement. *Chem. Eng. J.* 162, 935–940.
2490 <https://doi.org/10.1016/j.cej.2010.06.046>
- 2491 Ugwu, C.U., Aoyagi, H., 2012. Microalgal Culture Systems: An Insight into their Designs,
2492 Operation and Applications. *Biotechnology* 11, 127–132.
2493 <https://doi.org/10.3923/biotech.2012.127.132>
- 2494 Uquiche, E., Antilaf, I., Millao, S., 2016. Enhancement of pigment extraction from *B. braunii*
2495 pretreated using CO₂ rapid depressurization. *Braz. J. Microbiol.* 47, 497–505.
2496 <https://doi.org/10.1016/j.bjm.2016.01.020>
- 2497 Van Eykelenburg, C., 1977. On the morphology and ultrastructure of the cell wall of *Spirulina*
2498 *platensis*. *Antonie Van Leeuwenhoek* 43, 89–99. <https://doi.org/10.1007/BF00395664>
- 2499 Van Haver, L., Nayar, S., 2017. Polyelectrolyte flocculants in harvesting microalgal biomass
2500 for food and feed applications. *Algal Res.* 24, 167–180.
2501 <https://doi.org/10.1016/j.algal.2017.03.022>
- 2502 Vandamme, D., Beuckels, A., Markou, G., Foubert, I., Muylaert, K., 2015. Reversible
2503 Flocculation of Microalgae using Magnesium Hydroxide. *BioEnergy Res.* 8, 716–725.
2504 <https://doi.org/10.1007/s12155-014-9554-1>
- 2505 Vandamme, D., Foubert, I., Fraeye, I., Meesschaert, B., Muylaert, K., 2012. Flocculation of
2506 *Chlorella vulgaris* induced by high pH: role of magnesium and calcium and practical
2507 implications. *Bioresour. Technol.* 105, 114–119.
2508 <https://doi.org/10.1016/j.biortech.2011.11.105>
- 2509 Vandamme, D., Foubert, I., Muylaert, K., 2013. Flocculation as a low-cost method for
2510 harvesting microalgae for bulk biomass production. *Trends Biotechnol.* 31, 233–239.
2511 <https://doi.org/10.1016/j.tibtech.2012.12.005>
- 2512 van Spronsen, J., Cardoso, M.A.T., Witkamp, G.-J., de Jong, W., Kroon, M.C., 2011.
2513 Separation and recovery of the constituents from lignocellulosic biomass by using
2514 ionic liquids and acetic acid as co-solvents for mild hydrolysis. *Chem. Eng. Process.*
2515 *Process Intensif.* 50, 196–199. <https://doi.org/10.1016/j.cep.2010.12.010>
- 2516 Velasquez-Orta, S.B., Garcia-Estrada, R., Monje-Ramirez, I., Harvey, A., Orta Ledesma,
2517 M.T., 2014. Microalgae harvesting using ozoflotation: Effect on lipid and FAME
2518 recoveries. *Biomass Bioenergy* 70, 356–363.
2519 <https://doi.org/10.1016/j.biombioe.2014.08.022>
- 2520 Vergnes, J.B., Gernigon, V., Guiraud, P., Formosa-Dague, C., 2019. Bicarbonate
2521 Concentration Induces Production of Exopolysaccharides by *Arthrospira platensis*
2522 That Mediate Bioflocculation and Enhance Flotation Harvesting Efficiency. *ACS*
2523 *Sustain. Chem. Eng.* 7, 13796–13804.
2524 <https://doi.org/10.1021/acssuschemeng.9b01591>
- 2525 Wang, M., Yuan, W., 2016. Modeling bubble dynamics and radical kinetics in ultrasound
2526 induced microalgal cell disruption. *Ultrason. Sonochem.* 28, 7–14.
2527 <https://doi.org/10.1016/j.ultsonch.2015.06.025>
- 2528 Wang, Meng, Yuan, W., 2015. Microalgal cell disruption in a high-power ultrasonic flow
2529 system. *Bioresour. Technol.* 193, 171–177.
2530 <https://doi.org/10.1016/j.biortech.2015.06.040>
- 2531 Wang, M., Yuan, W., 2015. Microalgal Cell Disruption via Ultrasonic Nozzle Spraying. *Appl.*
2532 *Biochem. Biotechnol.* 175, 1111–1122. <https://doi.org/10.1007/s12010-014-1350-z>
- 2533 Wang, S., Yerkebulan, M., Abomohra, A.E.-F., El-Khodary, S., Wang, Q., 2019. Microalgae
2534 harvest influences the energy recovery: A case study on chemical flocculation of
2535 *Scenedesmus obliquus* for biodiesel and crude bio-oil production. *Bioresour. Technol.*
2536 286, 121371. <https://doi.org/10.1016/j.biortech.2019.121371>

- 2537 Wang, S., Huan J., Ke X., 2014. Effect of magnesium deficiency on photosynthetic
2538 physiology and triacylglyceride (TAG) accumulation of *Chlorella vulgaris*. *Biomass*
2539 *and Bioenergy*, 35, 1462–1467.
- 2540 Wang, W., Lee, D.-J., Lai, J.-Y., 2015. Aggregate formation affects ultrasonic disruption of
2541 microalgal cells. *Bioresour. Technol.* 198, 907–912.
2542 <https://doi.org/10.1016/j.biortech.2015.09.099>
- 2543 Weiss, N.D., Felby, C., Thygesen, L.G., 2019. Enzymatic hydrolysis is limited by biomass–
2544 water interactions at high-solids: improved performance through substrate
2545 modifications. *Biotechnol. Biofuels* 12, 3. <https://doi.org/10.1186/s13068-018-1339-x>
- 2546 Wu, C., Xiao, Y., Lin, W., Li, J., Zhang, S., Zhu, J., Rong, J., 2017. Aqueous enzymatic
2547 process for cell wall degradation and lipid extraction from *Nannochloropsis* sp.
2548 *Bioresour. Technol.* 223, 312–316. <https://doi.org/10.1016/j.biortech.2016.10.063>
- 2549 Wu, J., Liu, J., Lin, L., Zhang, C., Li, A., Zhu, Y., Zhang, Y., 2015. Evaluation of several
2550 flocculants for flocculating microalgae. *Bioresour. Technol.* 197, 495–501.
2551 <https://doi.org/10.1016/j.biortech.2015.08.094>
- 2552 Wu, X., Zhou, C., Li, K., Zhang, W., Tao, Y., 2018. Probing the fouling process and
2553 mechanisms of submerged ceramic membrane ultrafiltration during algal harvesting
2554 under sub- and super-critical fluxes. *Sep. Purif. Technol.* 195, 199–207.
2555 <https://doi.org/10.1016/j.seppur.2017.12.001>
- 2556 Xiao, C., Liao, Q., Fu, Q., Huang, Y., Chen, H., Zhang, H., Xia, A., Zhu, X., Reungsang, A.,
2557 Liu, Z., 2019. A solar-driven continuous hydrothermal pretreatment system for
2558 biomethane production from microalgae biomass. *Appl. Energy* 236, 1011–1018.
2559 <https://doi.org/10.1016/j.apenergy.2018.12.014>
- 2560 Xiaojian, D., Yun, H., Qiang, L., Qian, F., Ao, X., Chao, X., Xun, Z., Alissara, R., Zhidan, L.,
2561 2017. Medium-low temperature hydrothermal hydrolysis kinetic characteristics of
2562 concentrated wet microalgae biomass. *Int. J. Agric. Biol. Eng.* 10, 154–162.
2563 <https://doi.org/10.25165/ijabe.v10i1.2699>
- 2564 Xie, Y., Ho, S.-H., Chen, C.-N.N., Chen, C.-Y., Jing, K., Ng, I.-S., Chen, J., Chang, J.-S., Lu,
2565 Y., 2016. Disruption of thermo-tolerant *Desmodesmus* sp. F51 in high pressure
2566 homogenization as a prelude to carotenoids extraction. *Biochem. Eng. J.* 109, 243–
2567 251. <https://doi.org/10.1016/j.bej.2016.01.003>
- 2568 Xu, L., Wang, F., Li, H.-Z., Hu, Z.-M., Guo, C., Liu, C.-Z., 2010. Development of an efficient
2569 electroflocculation technology integrated with dispersed-air flotation for harvesting
2570 microalgae. *J. Chem. Technol. Biotechnol.* 85, 1504–1507.
2571 <https://doi.org/10.1002/jctb.2457>
- 2572 Xu, Y., Purton, S., Baganz, F., 2013. Chitosan flocculation to aid the harvesting of the
2573 microalga *Chlorella sorokiniana*. *Bioresour. Technol.* 129, 296–301.
2574 <https://doi.org/10.1016/j.biortech.2012.11.068>
- 2575 Xue, Y., Li, Y., Zou, X., Xu, K., Wen, H., Zhang, B., Li, R., Shao, P., Fu, B., Gong, Y., 2019.
2576 Optimization of thermal preflocculation treatment for effective air flotation harvesting
2577 of microalgae [WWW Document]. <https://doi.org/info:doi/10.1002/jctb.5941>
- 2578 Yap, B.H.J., Crawford, S.A., Dumsday, G.J., Scales, P.J., Martin, G.J.O., 2014. A
2579 mechanistic study of algal cell disruption and its effect on lipid recovery by solvent
2580 extraction. *Algal Res.* 5, 112–120. <https://doi.org/10.1016/j.algal.2014.07.001>
- 2581 Yap, B.H.J., Dumsday, G.J., Scales, P.J., Martin, G.J.O., 2015. Energy evaluation of algal cell
2582 disruption by high pressure homogenisation. *Bioresour. Technol.*, *Advances in*
2583 *biofuels and chemicals from algae* 184, 280–285.
2584 <https://doi.org/10.1016/j.biortech.2014.11.049>

2585 Ye, J., Sha, J., Liu, Q., Zhang, X., Hu, Q., Chen, Y., 2019. Influence of growth phase on the
2586 harvesting of *Scenedesmus acuminatus* using ultrafiltration. *Sci. Total Environ.* 660,
2587 25–31. <https://doi.org/10.1016/j.scitotenv.2019.01.020>

2588 Ye, Zhang., Tan, X.-H., Liu, Z.-W., Aadil, R.M., Tan, Y.-C., Inam-ur-Raheem, M., 2020
2589 Mechanisms of breakdown of *Haematococcus pluvialis* cell wall by ionic liquids,
2590 hydrochloric acid and multi-enzyme treatment. *Int. J. Food Sci. Technol.*
2591 <https://doi.org/10.1111/ijfs.14582>

2592 Yoo, G., Park, W.-K., Kim, C.W., Choi, Y.-E., Yang, J.-W., 2012. Direct lipid extraction
2593 from wet *Chlamydomonas reinhardtii* biomass using osmotic shock. *Bioresour.*
2594 *Technol.* 123, 717–722. <https://doi.org/10.1016/j.biortech.2012.07.102>

2595 Yoo, G., Yoo, Y., Kwon, J.-H., Darpito, C., Mishra, S.K., Pak, K., Park, M.S., Im, S.G.,
2596 Yang, J.-W., 2013. An effective, cost-efficient extraction method of biomass from wet
2597 microalgae with a functional polymeric membrane. *Green Chem.* 16, 312–319.
2598 <https://doi.org/10.1039/C3GC41695J>

2599 Yu, X., Zhao, P., He, C., Li, J., Tang, X., Zhou, J., Huang, Z., 2012. Isolation of a novel strain
2600 of *Monoraphidium* sp. and characterization of its potential application as biodiesel
2601 feedstock. *Bioresour. Technol.* 121, 256–262.
2602 <https://doi.org/10.1016/j.biortech.2012.07.002>

2603 Zakaria, S.M., Kamal, S.M.M., Harun, M.R., Omar, R., Siajam, S.I., 2017. Subcritical Water
2604 Technology for Extraction of Phenolic Compounds from *Chlorella* sp. *Microalgae and*
2605 *Assessment on Its Antioxidant Activity.* *Molecules* 22, 1105.
2606 <https://doi.org/10.3390/molecules22071105>

2607 Zbinden, M.D.A., Sturm, B.S.M., Nord, R.D., Carey, W.J., Moore, D., Shinogle, H., Staggs-
2608 Williams, S.M., 2013. Pulsed electric field (PEF) as an intensification pretreatment for
2609 greener solvent lipid extraction from microalgae. *Biotechnol. Bioeng.* 110, 1605–
2610 1615. <https://doi.org/10.1002/bit.24829>

2611 Zhang, B., Feng, H., He, Z., Wang, S., Chen, H., 2018. Bio-oil production from hydrothermal
2612 liquefaction of ultrasonic pre-treated *Spirulina platensis*. *Energy Convers. Manag.* 159,
2613 204–212. <https://doi.org/10.1016/j.enconman.2017.12.100>

2614 Zhang, R., Grimi, N., Marchal, L., Lebovka, N., Vorobiev, E., 2019a. Effect of
2615 ultrasonication, high pressure homogenization and their combination on efficiency of
2616 extraction of bio-molecules from microalgae *Parachlorella kessleri*. *Algal Res.* 40,
2617 101524. <https://doi.org/10.1016/j.algal.2019.101524>

2618 Zhang, R., Grimi, N., Marchal, L., Vorobiev, E., 2019b. Application of high-voltage electrical
2619 discharges and high-pressure homogenization for recovery of intracellular compounds
2620 from microalgae *Parachlorella kessleri*. *Bioprocess Biosyst. Eng.* 42, 29–36.
2621 <https://doi.org/10.1007/s00449-018-2010-4>

2622 Zhang, R., Marchal, L., Lebovka, N., Vorobiev, E., Grimi, N., 2020. Two-step procedure for
2623 selective recovery of bio-molecules from microalga *Nannochloropsis oculata* assisted
2624 by high voltage electrical discharges. *Bioresour. Technol.* 302, 122893.
2625 <https://doi.org/10.1016/j.biortech.2020.122893>

2626 Zhang, R., Parniakov, O., Grimi, N., Lebovka, N., Marchal, L., Vorobiev, E., 2019c.
2627 Emerging techniques for cell disruption and extraction of valuable bio-molecules of
2628 microalgae *Nannochloropsis* sp. *Bioprocess Biosyst. Eng.* 42, 173–186.
2629 <https://doi.org/10.1007/s00449-018-2038-5>

2630 Zhang, S., Gao, Y., Liu, Q., Ye, J., Hu, Q., Zhang, X., 2019. Harvesting of *Isochrysis*
2631 *zhanjiangensis* using ultrafiltration: Changes in the contribution ratios of cells and
2632 algogenic organic matter to membrane fouling under different cross-flow velocities.
2633 *Algal Res.* 41, 101567. <https://doi.org/10.1016/j.algal.2019.101567>

- 2634 Zhang, Y., Ward, V., Dennis, D., Plechkova, N.V., Armenta, R., Rehmann, L., 2018. Efficient
 2635 Extraction of a Docosaheptaenoic Acid (DHA)-Rich Lipid Fraction from
 2636 Thraustochytrium sp. Using Ionic Liquids. *Materials* 11, 1986.
 2637 <https://doi.org/10.3390/ma11101986>
- 2638 Zhao, F., Chu, H., Tan, X., Zhang, Y., Yang, L., Zhou, X., Zhao, J., 2016. Comparison of
 2639 axial vibration membrane and submerged aeration membrane in microalgae
 2640 harvesting. *Bioresour. Technol.* 208, 178–183.
 2641 <https://doi.org/10.1016/j.biortech.2016.02.099>
- 2642 Zhao, F., Chu, H., Zhang, Y., Jiang, S., Yu, Z., Zhou, X., Zhao, J., 2017. Increasing the
 2643 vibration frequency to mitigate reversible and irreversible membrane fouling using an
 2644 axial vibration membrane in microalgae harvesting. *J. Membr. Sci.* 529, 215–223.
 2645 <https://doi.org/10.1016/j.memsci.2017.01.039>
- 2646 Zhao, F., Zhang, Y., Chu, H., Jiang, S., Yu, Z., Wang, M., Zhou, X., Zhao, J., 2018. A
 2647 uniform shearing vibration membrane system reducing membrane fouling in algae
 2648 harvesting. *J. Clean. Prod.* 196, 1026–1033.
 2649 <https://doi.org/10.1016/j.jclepro.2018.06.089>
- 2650 Zhu, Y., Dunford, N.T., Goad, C., 2014. Effect of Processing Parameters on Flocculation of
 2651 *Picochlorum oklahomensis*. *J. Am. Oil Chem.*
 2652 *Soc.* 91, 317–324. <https://doi.org/10.1007/s11746-013-2371-4>
- 2653 Zuorro, A., Maffei, G., Lavecchia, R., 2016. Optimization of enzyme-assisted lipid extraction
 2654 from *Nannochloropsis* microalgae. *J. Taiwan Inst. Chem. Eng.* 67, 106–114.
 2655 <https://doi.org/10.1016/j.jtice.2016.08.016>

2657
 2658
 2659
 2660
 2661
 2662

Table 1. Effects of medium composition on the chemical composition of various microalgae species.
 Table adapted from Juneja et al 2013. Information on magnesium from (Wang. et al. 2014, Polat et al.
 2020).

2663

2664

2665

Medium composition	Conditions	Microalgae specie	Biochemical modification
Nitrogen	Limitation	<i>Nannochloropsis oculata</i>	Increase in lipid synthesis
		<i>Phaeodactylum tricornutum</i>	Increase in lipid synthesis and decrease in protein content
		<i>Chlorella vulgaris</i>	Increase in lipid synthesis
		<i>Haematococcus pluvialis</i>	Increase in carotenoid formation
Phosphorus	Limitation	<i>Chlamydomonas reinhardtii</i>	Decrease in phosphatidylglycerol
	Limitation	<i>Ankistrodesmus falcatus</i>	Decrease in chlorophyll and protein; Increase in carbohydrate and lipids
	Starvation	<i>Selenastrum minutum</i>	Reduced rate of respiration; Decreased photosynthetic CO ₂ fixation
Iron	Limitation	<i>Dunaliella tertiolecta</i>	Decrease in cellular chlorophyll concentration
	High concentration	<i>Chlorella vulgaris</i>	Increase in lipid content
	High concentration	<i>Haematococcus pluvialis</i>	Increase in carotenoid formation
Carbon	Increasing CO ₂ concentration at 10%	<i>Chlamydomonas reinhardtii</i>	Increase in amount of fatty acid
	Increasing CO ₂ concentration at 10%	<i>Dunaliella salina</i>	Increase in amount of fatty acid
	Elevated CO ₂ concentrations	<i>Spirulina platensis</i>	Increase in carbohydrate content; Decrease in proteins and pigments
Salinity	Increasing NaCl concentration	<i>Dunaliella</i> sp.	Increase in saturated and monounsaturated fatty acids
	Increasing NaCl concentration	<i>Dunaliella tertiolecta</i>	Increase in intracellular lipids and triglyceride concentration
	Increasing NaCl concentration	<i>Botryococcus braunii</i>	Increase in carbohydrate content, and lipid content. Decrease of protein content
Magnesium	Magnesium deficiency	<i>Chlorella Vulgaris</i>	Decrease chlorophyll and protein. Increase in triacylglyceride (TAG) accumulation
	Magnesium starvation	<i>Auxenochlorella protothecoides</i>	Decrease the total chlorophyll production. Increase the lipid content

Table 2. Cell wall structures and high-value products of typical microalgae species

Robustness of cell wall	Species	Cell size (μm)	Cell wall composition	Reference	Main product/price ^a
High	<i>Scenedesmus</i> sp.	5-7	Trilaminar sheath (algaenan), Inner cell wall (mannose, glucose), coenobial adhesive	(Baudelet et al., 2017).	Carbohydrates
High	<i>Haematococcus pluvialis</i>	20	Trilaminar sheath (algaenan), primary, secondary and tertiary cell wall (mannose, other sugars)	(Baudelet et al., 2017).	Astaxanthin / 1,450-5075 € kg ⁻¹
Medium	<i>Phaeodactylum tricornutum</i>	25	Silica, sulfated α -glucuronomannan, proteins, polyamines	(Johansen, 1991) (Le Costaouëc et al., 2017)	Fucoanthin/ \$ 200 kg ⁻¹ -4450 €g ⁻¹ ^b
Medium	<i>Arthrospira platensis</i>	8	Peptidoglycan layer (Murein), two fibrillary layers, sheath of acidic polysaccharides	(Akao et al., 2019; Kröger et al., 2018)	Phycocyanin/360€ kg ⁻¹
Medium	<i>Chloromonas nivalis</i>	15	By-layered cell wall	(Remias et al., 2010)	Astaxanthin/ 1,450-5075 € kg ⁻¹
Medium	<i>Dunaliella salina</i>	5-25	Double-layered rigid wall (hyaline, algaenan)	(Baudelet et al., 2017)	β -carotene/ 215-2150 € kg ⁻¹
Low	<i>Isochrysis galbana</i>	5–6	Lack of cell wall, plasma membrane	(Thu et al., 2015)	ω 3 ω 6/ 78-116 € kg ⁻¹

^a as reviewed in (Cuellar-Bermudez et al., 2015), ^b from (Shannon and Abu-Ghannam, 2018)

Table 3. Flocculation process parameters for typical polyelectrolytes. Table adapted from Van Haver and Nayar, 2017

Flocculant	Cell concentration	Dosage (mg L ⁻¹)	Volume (L)	Time (min)	Species	Harvesting efficiency (%)	Reference
Chitosan	-	20	0.5	240	<i>C. calcitrans</i>	98	Harith et al. 2009
Magnafloc® LT 25	-	0.1	0.5	240	<i>C. calcitrans</i>	83	Harith et al. 2009
Chitosan+Sodium alginate	1.24 ^a	30 ppm + 40 ppm	0.01	60	<i>S. obliquus</i>	90	Matter et al., 2018
Zetag 7557, Polyacrylamide	0.7 ^b	10	0.004	120	<i>Neochloris oleoabundans</i>	52	Lam, 2017
Synthofloc 5080H, Polyacrylamide	3.6 ^b	10	0.25	120	<i>P. tricornutum</i>	95	Lam, 2017
Cationic starch	0.77 g L ⁻¹	40	-	30	<i>C. protothecoides</i>	90	Letelier-Gordo et al., 2014
Zetag 63, Polyacrylamide	10 ⁶ cells mL ⁻¹	10	1	30	<i>Chlorella stimatophora</i>	93	Van Haver and Nayar, 2017
F04650, Polyacrylamide, Medium charge	0.7 g L ⁻¹	3	0.05	60	<i>N. salina</i>	73	Van Haver and Nayar, 2017
FO4800, Polyacrylamide, High charge	0.7 g L ⁻¹	3	0.05	60	<i>N. salina</i>	88	Van Haver and Nayar, 2017

FO4990, Polyacrylamide, Very high charge	0.7 g L ⁻¹	3	0.05	60	<i>N. salina</i>	94	Van Haver and Nayar, 2017
Emfloc KC750, Potato starch	10 ⁷ cells mL ⁻¹	70	0.1	30	<i>C. reinhardtii</i>	90	Van Haver and Nayar, 2017
POLYSEPAR®CFL25, Tannin,quaternary ammonia salt	10 ⁷ cells mL ⁻¹	30	0.1	30	<i>Chlorella sp.</i>	95	Van Haver and Nayar, 2017
Tanfloc SL, Tannin Natural polymer	0.26	5	3	-	<i>C. vulgaris</i>	100	Van Haver and Nayar, 2017

OD: Optical Density

^a OD_{680 nm}

^b OD_{750 nm}

Table 4. Process parameters and yields of typical microalgae harvesting processes

Method	Species	Time	Initial biomass concentration	Total volume	Harvesting efficiency	Reference
Gravitational settling	<i>Monoraphidium</i> sp. FXY-10	24 h	0.218 g L ⁻¹	-	98 %	Yu et al., 2012
Gravitational settling	<i>A. platensis</i>	-	0.55 g L ⁻¹	1 L	85 %	Depraetere et al., 2015
Coagulation	<i>N. salina</i>	2.5 h	0.5 g L ⁻¹	0.113 L	85 %	Chatsungnoen and Chisti, 2016
Flocculation	<i>C. reinhardtii</i>	0.25 h	0.7 g L ⁻¹	0.1 L	90%	Fan et al., 2017
Flocculation	<i>C. calcitrans</i>	0.17 h	0.095 g L ⁻¹	0.25 L	89 %	Şirin et al., 2015
Flocculation	<i>C. vulgaris</i>	0.5 h	0.5 g L ⁻¹	0.1 L	90 %	Vandamme et al., 2012
Flocculation	<i>D. brasiliensis</i>	0.017 h	0.5 g L ⁻¹	50 L	99.2 %	Ndikubwimana et al., 2016
Centrifugation	<i>A. platensis</i>	0.17 h	0.41 g L ⁻¹	0.005 L	85%	Cuellar-Bermudez et al., 2019
Centrifugation	<i>S. obliquus</i>	0.17 h	0.746 g L ⁻¹	70 L	99.3%	Wang et al., 2019
Dissolved air flotation	<i>Mixed microalgae</i>	-	0.545 g L ⁻¹	-	91%	Niaghi et al., 2015
Flocculation-flotation	<i>S. obliquus</i>	0.25 h	0.73x10 ⁶ cells mL ⁻¹	1 L	93.6 %	Xue et al., 2019
Bioflocculation	<i>A. plantensis</i>	0.25 h	1.4 g L ⁻¹	0.6 L	95%	Vergnes et al., 2019
Bioflocculation	<i>Mixed microalgae</i>	0.083 h	0.419 g L ⁻¹	0.95 L	79.6%	Velasquez-Orta et al., 2014

Table 5. Overview of major microalgae cell disruption methods

Method	Mechanism	Advantages	Disadvantages	Cell concentration	Selectivity product separation	Energy requirements
Bead Milling	Beads collision (cell cleavage, fracture, abrasion)	Effective cell disruption High biomass concentration	Emulsification of products Requires cooling	High	Low	Low
High-pressure homogenization	shear stress induced pressure drop	Effective cell disruption High biomass concentration Viscosity reduction	Emulsification of products may hamper separation	High	Low	Low
Ultrasonication	Shear force from bubble cavitation Free radical formation/oxidation	Simple Can be combined with other processes	Low disruption efficiency Low-medium biomass concentration Requires cooling	Low-medium	Low	Low
Pulsed Electric Field	Electroporation of cell wall/membrane	Mild effect High biomass concentration	Inefficient for small cell size	High	Medium	Low

Hydrothermal/Steam Explosion	Explosion of cells from pressure drop Hydrolysis of cell wall components	Lack of reagents Viscosity reduction due to depolymerization of macromolecules Simple reactor set-up	Degradation of sensitive products Generation of fermentation inhibitors Hydrolysis of carbohydrates & protein may be undesirable	Medium-High	Low	Medium
Acid treatment	Hydrolysis of cell wall components	Effective cell disruption Simple reactor set-up	Neutralization of process liquid Degradation of sensitive products Generation of fermentation inhibitors	Medium-High	Low	Medium
Alkaline treatment	Hydrolysis of cell wall components	Effective cell disruption Simple reactor set-up	Neutralization of process liquid Degradation of sensitive products Generation of fermentation inhibitors	Medium-High	Low	Medium

			Long treatment time			
Ozonolysis	Oxidation of cell wall components	Optimal for biodiesel production	Oxidation of sensitive & high-value products	Low	Low	Low
Hydrogen peroxide	Oxidation of cell wall components	Optimal for biodiesel production	Oxidation of sensitive & high-value products	Low	Low	Low
Enzymatic	Hydrolysis/ Oxidation of cell wall components	High specificity Low temperature No degradation	High enzyme cost Long process time	Medium	High	Low

Table 6. Effect of different drying methods on extraction of biomolecules from *S. obliquus*. Table adapted from Ansari et al., 2018.

Drying Method	Process conditions	Lipid yield (% w/w)	Protein yield (% w/w)	Carbohydrate yield (% w/w)	Energy (KWh kg⁻¹)	Cost	Reference
Untreated	-	-	31.07	19.9	-	-	Ansari et al., 2018
Sun-drying	Natural sunlight (400-1200 mmolm ⁻² s ⁻¹), 18-27 °C, 2-3 days	11.64	25.7	19.5	0.055	0.011 \$ L ⁻¹	Ansari et al., 2018
Freeze-drying	Deep freeze -84 °C , 0.9 kW for 24 h	18.45	31	21.3	21.81	1.189 \$ L ⁻¹	Ansari et al., 2018
Oven-drying	Air oven, 60 °C 12 h	13	30.98	18.8	6.33	0.343 \$ L ⁻¹	Ansari et al., 2018
Spray dryer	-	-	-	-	6.1	0.30 € kg ⁻¹	Fasaei et al. 2018
Drum dryer	-	-	-	-	5.1	0.25 € kg ⁻¹	Fasaei et al. 2018

Table 7. Effect of process severity factor during hydrothermal pretreatment on biomolecule extraction yields

Species	Pretreatment	Reactor loading/ Biomass	Time	Temperature	Yield		
	method	Concentration	(min)	(°C)	<i>logR_o</i>	(%)	Reference
<i>Lipids</i>							
<i>Chlorella sorokiniana</i>	Steam explosion	100g / 20% w/w solids	5	120	1.29	18.4 ^a	Lorente et al., 2015
<i>Nannochloropsis gaditana</i>	Steam explosion	250g / 17% w/w solids	5	120	1.29	3.6 ^a	Nurra et al., 2014
<i>Nannochloropsis gaditana</i>	Steam explosion	100g / 20% w/w solids	5	120	1.29	17.9 ^a	Lorente et al., 2015
<i>Nannochloropsis gaditana</i>	Steam explosion	100g / 20% w/w solids	5	150	2.17	18.2 ^a	Lorente et al., 2015
<i>Chlorella vulgaris</i>	Hydrothermal	30 mL/ 50 g L ⁻¹	120	150	3.60	67.7 ^c	Xiaojian et al., 2017
<i>Nannochloropsis oceanica</i>	Steam explosion	200 mL / 10% w/w solids	5	207.12	3.85	93.9 ^b	Cheng et al., 2015
<i>Nannochloropsis sp.</i>	Hydrothermal	na ^e / 20 w/w solids	60	180	4.13	84.0 ^d	Qu et al., 2018
Carbohydrates							
<i>Nannochloropsis gaditana</i>	Steam explosion	100g / 20% w/w solids	5	120	1.29	43.7 ^f	Lorente et al., 2015
<i>Chlorella vulgaris</i>	Hydrothermal	na ^e / 5g tCOD L ⁻¹	20	120	1.89	51.8 ^f	Mendez et al., 2013
<i>Nannochloropsis gaditana</i>	Steam explosion	100g / 20% w/w solids	5	150	2.17	44.4 ^f	Lorente et al., 2015
<i>Chlorella vulgaris</i>	Hydrothermal	na ^e / 5g tCOD L ⁻¹	40	120	2.19	58.2 ^f	Mendez et al., 2013
<i>Chlorella pyrenoidosa</i>	Hydrothermal	na ^e / 10 % w/w solids	10 ^g	160	2.77	46.9	Fu et al., 2018
<i>Chlorella pyrenoidosa</i>	Hydrothermal	na ^e / 7 % w/w solids	15 ^h	155.2	2.80	80.5	Xiao et al., 2019

<i>Chlorella vulgaris</i>	Hydrothermal	30 mL/ 50 g L ⁻¹	120	150	3.55	60.6	Xiaojian et al., 2017
Protein							
<i>Chlorella vulgaris</i>	Hydrothermal	na ^e / 5g tCOD L ⁻¹	20	120	1.89	20.2 ⁱ	Mendez et al., 2013
<i>Chlorella vulgaris</i>	Hydrothermal	na ^e / 5g tCOD L ⁻¹	40	120	2.19	26.4 ⁱ	Mendez et al., 2013
<i>Chlorella pyrenoidosa</i>	Hydrothermal	na ^e / 10 % w/w solids	10	160	2.77	46.9 ^j	Fu et al., 2018
<i>Chlorella vulgaris</i>	Hydrothermal	30 mL/ 50 g L ⁻¹	60	200	4.72	75.9 ⁱ	Xiaojian et al., 2017

^aBligh and Dyer method : chloroform–methanol (1:2 v/v); ^b modified Bligh and Dyer method ; ^chexane-methanol (7:3 v/v); ^d diethyl ether ^e na : information not available.; ^fcalculated as % of total monosaccharides measured in the pretreatment liquid; ^g retention time in a continuous system with a flow rate of 14 L h⁻¹; ^h retention time in a continuous system with a flow rate of 40 L h⁻¹; ⁱ calculated as % of total protein measured in the pretreatment liquid as total Kjeldahl nitrogen (TKN) x 5.95 ; ^j calculated as % of total protein measured in the pretreatment liquid with the Lowry method

Table 8. Effect of process severity factor during dilute acid pretreatment on biomolecule extraction yields

Species	Acid	Reactor loading/ Biomass		Time (min)	Temperature (°C)	Yield (%)	Reference
		Concentration	CSF				
Lipids							
<i>N. gaditana</i>	H ₂ SO ₄ , 5% w/w wet basis	100g / 20% w/w solids		5	120	0.40	17.7 ^a Lorente et al., 2015
<i>N. gaditana</i>	H ₂ SO ₄ , 10 % w/w wet basis	100g / 20% w/w solids		5	120	0.75	17.8 ^a Lorente et al., 2015
<i>N. gaditana</i>	H ₂ SO ₄ , 5% w/w wet basis	100g / 20% w/w solids		5	150	1.02	18.5 ^a Lorente et al., 2015
<i>N. salina</i>	HNO ₃ 0.57%, v/v	na/ 20 g L ⁻¹		30	120	1.21	24.4 ^b Lee et al., 2014
<i>N. gaditana</i>	H ₂ SO ₄ , 10 % w/w wet basis	100g / 20% w/w solids		5	150	1.40	18.5 ^a Lorente et al., 2015
<i>N. gaditana</i>	H ₂ SO ₄ , 5% w/w wet basis	100g / 20% w/w solids		5	150	1.87	79 ^a E. Lorente et al., 2017
<i>N. salina</i>	HNO ₃ 0.5%, v/v	na/ 20 g L ⁻¹		60	120	1.94	88.4 ^b Lee and Han, 2015
Carbohydrates							
mixed culture	H ₂ SO ₄ 0.5 M	na		10	121	1.32	18 ^c Shokrkar et al., 2017
mixed culture	H ₂ SO ₄ 0.5 M	na		20	121	1.62	44 ^c Shokrkar et al., 2017
mixed culture	H ₂ SO ₄ 1 M	na		20	121	1.92	58 ^c Shokrkar et al., 2017
mixed culture	H ₂ SO ₄ 0.5 M	na		40	121	1.92	85 ^c Shokrkar et al., 2017
mixed culture	H ₂ SO ₄ 1 M	na		40	121	2.22	92 ^c Shokrkar et al., 2017

mixed culture	H ₂ SO ₄ 2 M	na	40	121	2.52	94 ^c	Shokrkar et al., 2017
---------------	------------------------------------	----	----	-----	------	-----------------	-----------------------

^aBligh and Dyer method : chloroform–methanol (1:2 v/v); ^b hexane; ^ctotal reducing sugar content of pretreatment liquid determined with dinitrosalicylic acid method

Table 9. Extraction yield achieved by different cell disruption methods

Cell disruption method	Species	Process parameters			Yield (%)	Extraction method	Reference
		Time (min)	Temperature (°C)	Process specific			
<i>Lipids</i>							
Bead milling	<i>C. vulgaris</i>	60	33	0.3–0.5 mm Y ₂ O ₃ stabilized ZrO ₂ grinding beads. 1:13 solid:water ratio (w v ⁻¹)	67	SC-CO ₂ 600 bar, 30 g min ⁻¹ CO ₂ flow rate, 5% ethanol, 60 °C	Safi et al., 2014
High-pressure homogenization	<i>Chlorella sp</i>	-	-	Single pass at 1400 bar	100	modified Bligh and Dyer, 20 min solvent contact time	Yap et al., 2014
Pulsed Electric Field	<i>A.protothecoides</i>	-	-	35 kV cm ⁻¹ 100 g L ⁻¹ algae slurry, 6 mL min ⁻¹	220 mg g ⁻¹	100% ethanol	Eing et al., 2013
Hydrothermal	<i>Nannochloropsis sp.</i>	60	180	20 w/w solids	84.0 ^d	diethyl ether	Qu et al., 2018

Dilute acid	<i>N. salina</i>	60	120	HNO ₃ 0.5% v/v, 20 g L ⁻¹ algae slurry	88.4	hexane	Lee and Han, 2015
Alkaline	<i>Nannochloropsis</i> <i>sp.</i>	480	100	300 g L ⁻¹ algae slurry, pH 10.5 (adjusted by 2mM NaOH)	83.5	30 mL chloroform	Wu et al., 2017
Oxidation	<i>Chlorella sp.</i> KR-1	60	90	17 g L ⁻¹ algae slurry, 2 mM K ₂ S ₂ O ₈	95	1h extraction with 5mL chloroform	Seo et al., 2016
Enzymatic	<i>Nannochloropsis</i> <i>sp.</i>	210	53	20 g L ⁻¹ algae slurry, pH 4.4, cellulase: 13.8 mg g ⁻¹ biomass, Endo- β-1,4- mannanase: 1.5 mg g ⁻¹ biomass	90	1 h Extraction with chloroform/methanol (2:1, v/v) and stirred at 37 °C	Zuorro et al., 2016
Carbohydrate							

Bead milling	<i>T. suecica</i>	15	25	Yttrium stabilized ZrO ₂ 0.3 mm beads, bead filling ratio 65% v/v,	99	Determined in the supernatant by the phenol - sulfuric acid assay	Postma et al., 2017
High-pressure homogenization	<i>C. vulgaris</i>	-	< 25 °C	5 passes 1500 bar, at 155 mL min ⁻¹ , 12 g L ⁻¹ biomass slurry	41.9% (w/w)	Determined in the supernatant by the DuBois method	Carullo et al., 2018
Hydrothermal	<i>C. pyrenoidosa</i>	15 ^h	155	7 % w/w solids	80.5	Determined in the supernatant by the phenol-sulfuric acid method	Xiao et al., 2019
Dilute acid	mixed culture	40	121	H ₂ SO ₄ 2 M	94 °C	determined in the pretreatment liquid with dinitrosalicylic acid method	Shokrkar et al., 2017

Alkaline	<i>H. tetrachotoma</i> <i>ME03</i>	30	121	10% w/v lyophilized microalgae slurry, 2M NaOH	80	determined in the pretreatment liquid anthrone method	Onay 2019
Protein							
Bead milling	<i>T. suecica</i>	6,67	25	Yttrium stabilized ZrO ₂ 0.3 mm beads, bead filling ratio 65% v/v,	99	Determined in the supernatant by the Lowry assay	Postma et al., 2017
High-pressure homogenization	<i>C. vulgaris</i>	-	< 25 °C	5 passes 1500 bar, at 155 mL min ⁻¹ , 12 g L ⁻¹ biomass slurry	54.1%	Determined in the supernatant by the Lowry assay	Carullo et al., 2018
Hydrothermal	<i>C. vulgaris</i>	60	200	30 mL/ 50 g L ⁻¹	75.9 ^j		Xiaojian et al., 2017
Steam explosion Dilute acid	<i>C.sorokiniana</i>	5	150	4 kg microalgae impregnated with H ₂ SO ₄ at a concentration of 5% w/w wet sample basis	55.7	determined in the pretreatment liquid with the bicinchoninic acid protein assay	Lorente et al., 2018

Alkaline	<i>C. vulgaris</i>	40	120	25 g tCOD L ⁻¹ biomass slurry, pH 10 (adjusted by 4M NaOH)	42	total Kjeldahl nitrogen x 5.95	Mendez et al., 2013
Pigments							
Ball mill grinding	<i>C. zofingiensis</i>	3.5	-	10 mm stainless steel balls	100	Acetone extraction of lutein	Araya et al., 2014
High-pressure homogenization	<i>Desmodesmus</i> <i>sp. F51</i>	-	-	2413 bar, 4 passes at 40 mL min ⁻¹	7.9 mg g ⁻¹ biomass	carotenoids extracted by ethyl ether	Xie et al., 2016
Pulsed Electric Field	<i>H. pluvialis</i>	-		1 kV·cm ⁻¹ , 10 pulses, 50 ms,	96	6h post treatment incubation followed by extraction of astaxanthin with 99.5% v/v methanol	Martínez et al., 2019
Hydrothermal	<i>H. pluvialis</i>	10	200	high temperature and high pressure microfluidic platform	100	20 min acetone extraction of astaxanthin	Cheng et al., 2017

Dilute acid	<i>H. pluvialis</i>	60	70	4M HCl, 10 g l ⁻¹ algae slurry	80	60 min acetone extraction of astaxanthin	Liu et al., 2018
Alkaline	<i>S. almeriensis</i>	80	20	100 g L ⁻¹ algae slurry, 4 w/v % KOH	99	5 min hexane extraction of lutein	Cerón et al., 2008

Table 10. Disruption and lipid extraction from *C. vulgaris* cells by various imidazolium based ionic liquids. Table adapted from (Orr et al., 2016).

Species	Ionic liquid	Algae/ionic liquid ration	Time (min)	Temperature (°C)	Cell disruption^a	Lipid yield (% total lipids)
<i>C. vulgaris</i>	1-ethyl-3-methylimidazolium chloride	1 : 20	30	140	Complete lysis	96
<i>C. vulgaris</i>	1-butyl-3-methylimidazolium chloride	1 : 20	30	140	Complete lysis	88
<i>C. vulgaris</i>	1-(2-hydroxyethyl)-3-methylimidazolium chloride	1 : 20	30	140	Complete lysis	98
<i>C. vulgaris</i>	1-allyl- 3-methylimidazolium chloride	1 : 20	30	140	Complete lysis	45

^a microscopic observation

Table 11. Energy and cost requirements of typical microalgae harvesting methods. Table adapted from Fasaei et al., 2018

Harvesting method	Size or processing capacity	Costs	Concentration of biomass (kg m ⁻³)	Energy requirement (kWh m ⁻³)	Biomass recovery (%)	Output solid concentration (%)
Centrifuge	80 m ³ ·h ⁻¹	250,000 €	200	0.70-1.30	95-99	10-20
Microfiltration	-	300 € m ⁻²	50	-	-	-
Flocculation	100 m ³	125,000	-	-	-	-
Spiral plate	4 m ³ h ⁻¹	229,000	200	0.95-2.00	95–99	20–22
Dissolved air flotation	3.8 L	-	0.545	0.0025	91	-
Membrane filtration	-	-	-	0.80-2.51	99	1.5-10
Electroflocculation	1.5 L	-	0.3-0.6	0.15-1	80–95	-
Flocculation + Centrifuge	-	0.98 € Kg ⁻¹	-	0.06 kWh·kg ⁻¹	-	-
Flocculation + Membrane filter	-	0.97 € Kg ⁻¹	-	0.05 kWh·kg ⁻¹	-	-

Membrane filter + Centrifuge	-	0.30 € Kg ⁻¹	-	0.84 kWh·kg ⁻¹	-	-
---------------------------------	---	-------------------------	---	---------------------------	---	---

Table 12. Energy and cost requirements of cell disruption methods

Disruption method	Species	Biomass concentration	Energy (KWh kg⁻¹)	Cost	Reference
HPH	<i>N. gatidana</i>	100 g L ⁻¹	0.32	0.15-0.25 ^a	Safi et al. 2017
Bead milling	<i>N. gatidana</i>	100 g L ⁻¹	0.43	0.4-1 ^a	Safi et al. 2017
PEF	<i>N. gatidana</i>	100 g L ⁻¹	10.42	2 - 20 ^a	Safi et al. 2017
Enzymatic	<i>N. gatidana</i>	100 g L ⁻¹	0.34	-	Safi et al. 2017
Ultrasound	<i>S. obliquus</i>	-	1.5	0.26 ^b	Ansari et al., 2018
Autoclave	<i>S. obliquus</i>	-	1.58	0.26 ^b	Ansari et al., 2018
Steam explosion	<i>N. oceanica</i>	10 % w/w	2.26	-	Cheng et al., 2015
Osmotic shock	<i>S. obliquus</i>	-	1.72	0.31 ^b	Ansari et al., 2018

^a € Kg⁻¹ of isolated protein ^b € L⁻¹ of treated sample

Figure captions

Figure 1. Overview of microalgae biorefinery

Figure 2. Cell wall structure of a: A *Chlamydomonas* biflagellate cell, B *Desmodesmus* four-celled coenobium, C mature *Haematococcus lacustris* cystm from (Baudalet et al., 2017), D *Phaeodactylum tricornutum* from (Gügi et al., 2015), E *Arthrospira platensis* (Van Eykelenburg, 1977) F *Tisochrysis lutea* (Orlova et al., 2019).

Figure 3. Typical mechanisms of microalgae flocculation (a) coagulation with bivalent cations (b) neutralization and bridging effect of cationic polymers (c) autoflocculation induced by excreted algogenic organic matter (d) detailed mechanism of flocculation with cationic polymers including “charge shielding” hypothesis for negative effect of high cationic polymer dosages (adapted from Lam, 2017)

Figure 4. Types of forces acting acting on microalgae cellss during bead milling. The diagram is not to scale and served for illustration purposes only.

Figure 5. Mechanism of cell disruption during High Pressure Homogenization. The cell sizedigram is not shown to scale. Pressure drop is the main effect of the process causingcause of the cell disruption.

Figure 6. Mechanism of cell disruption by ultrasound treatment

Figure 7. Pore formation in the cell membrane and subsequent release of intracellular molecules as a result of PEF treatment of microalgae cells

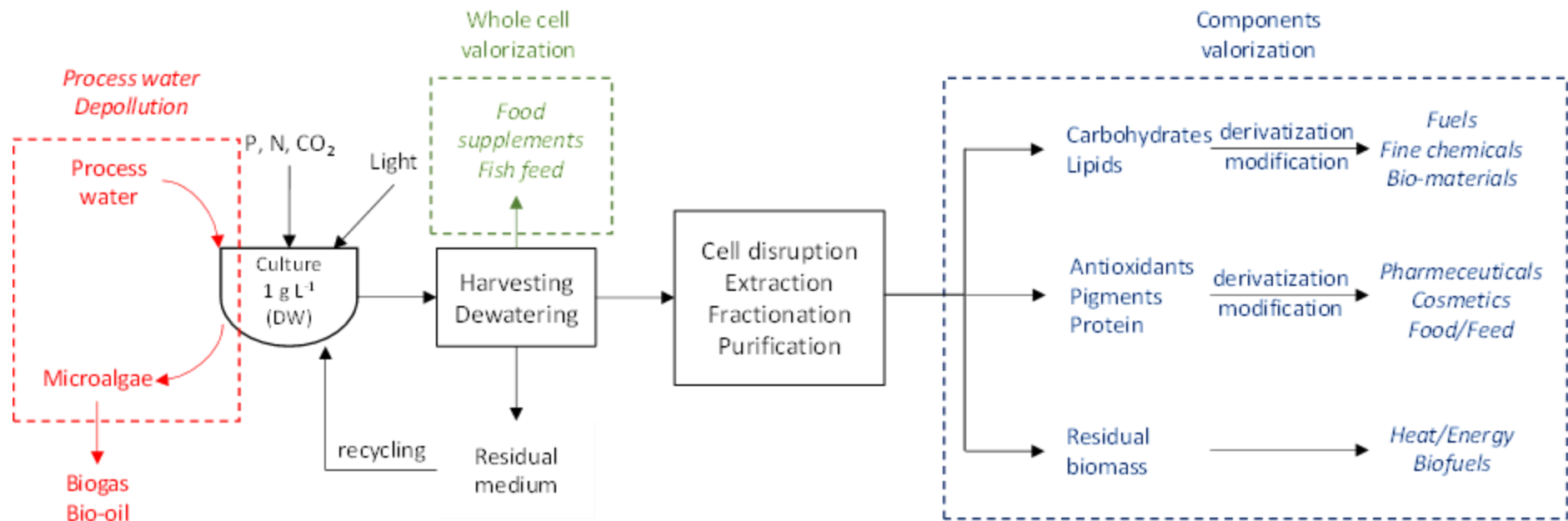


Figure 1

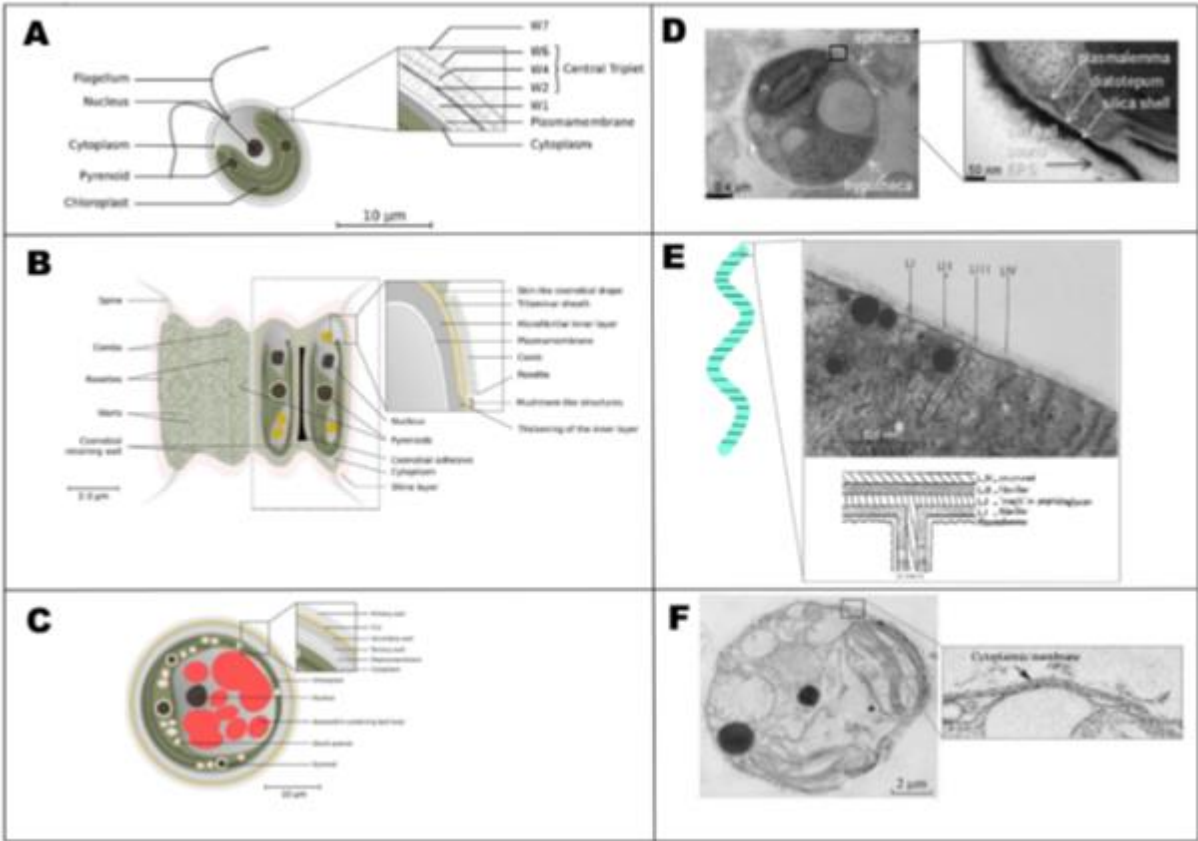


Figure 2

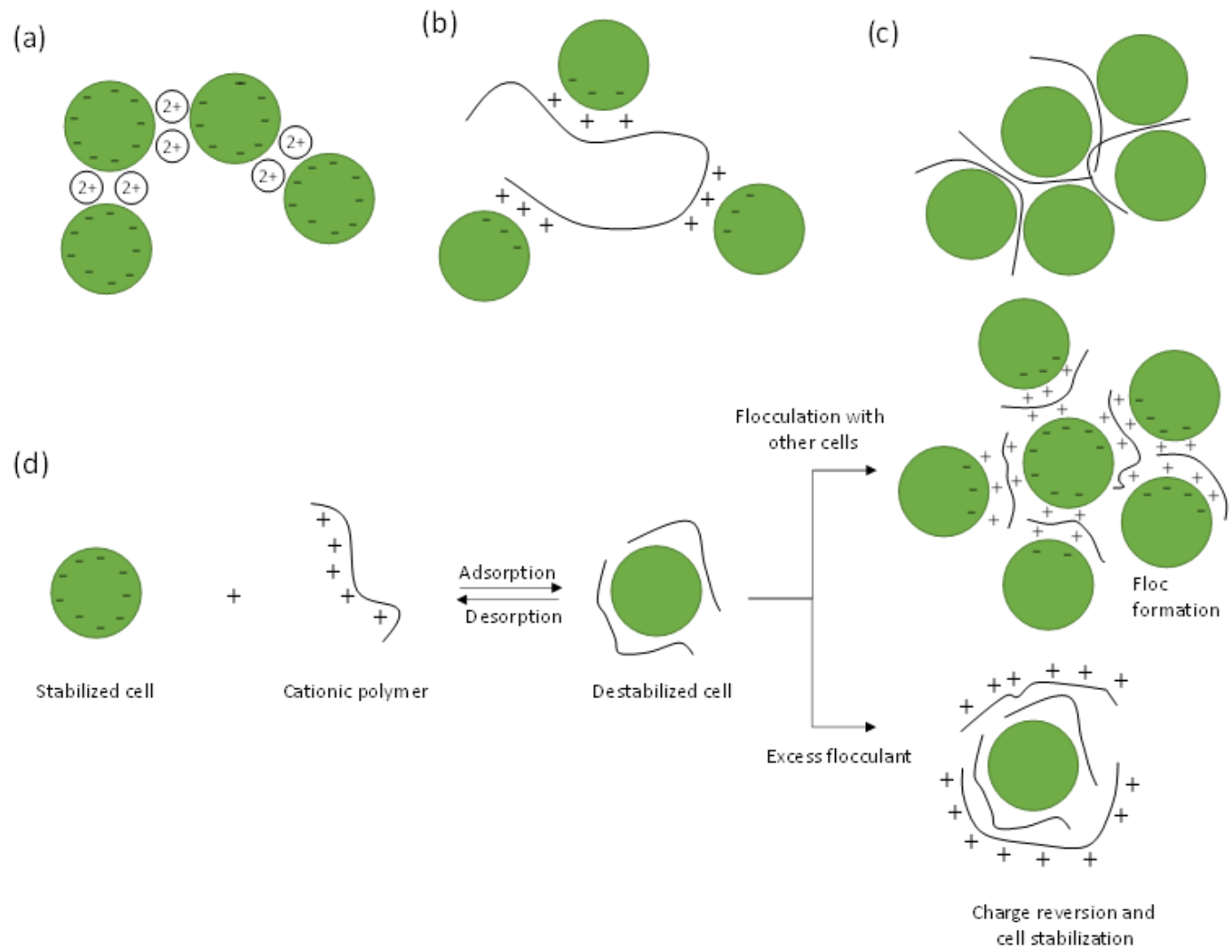


Figure 3

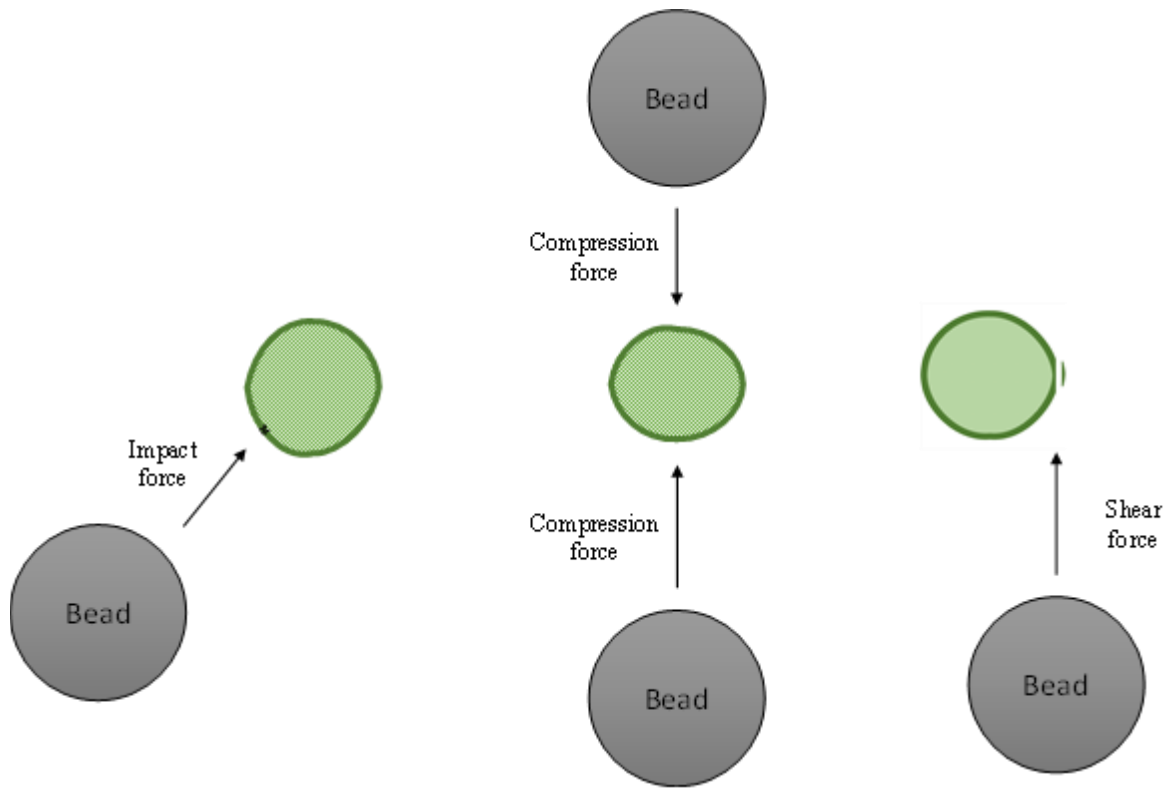


Figure 4

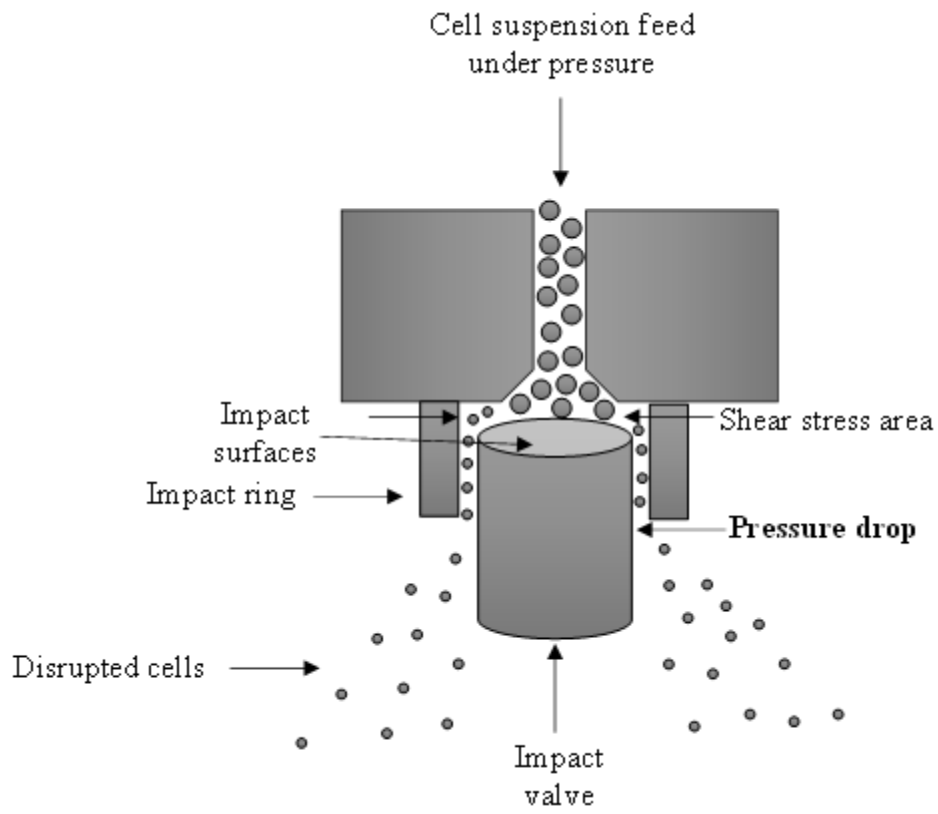


Figure 5

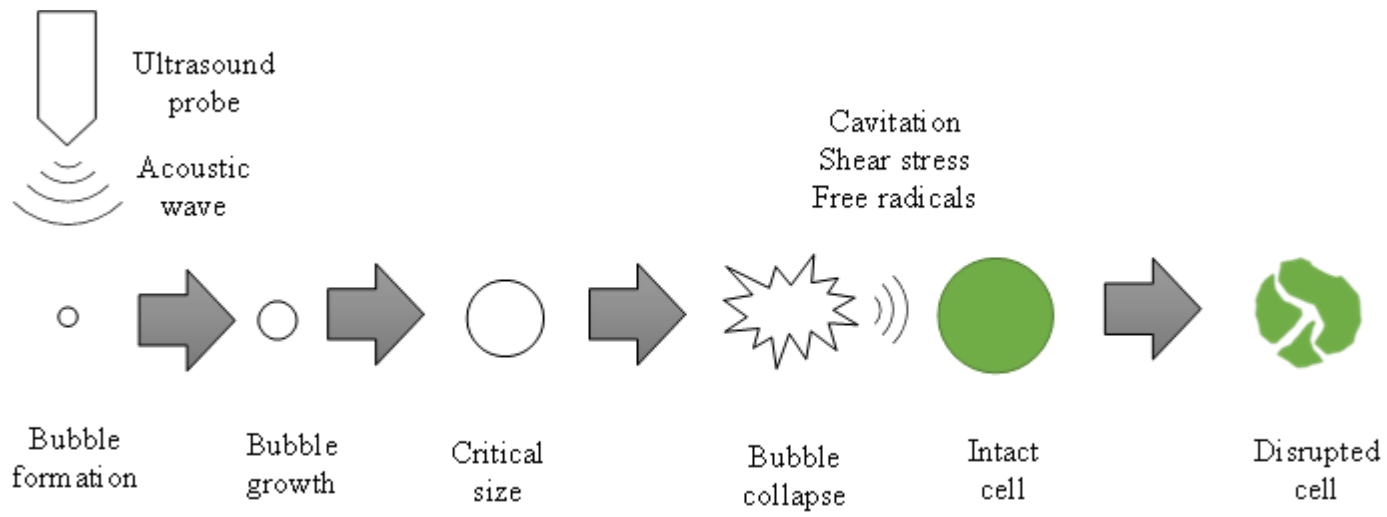


Figure 6

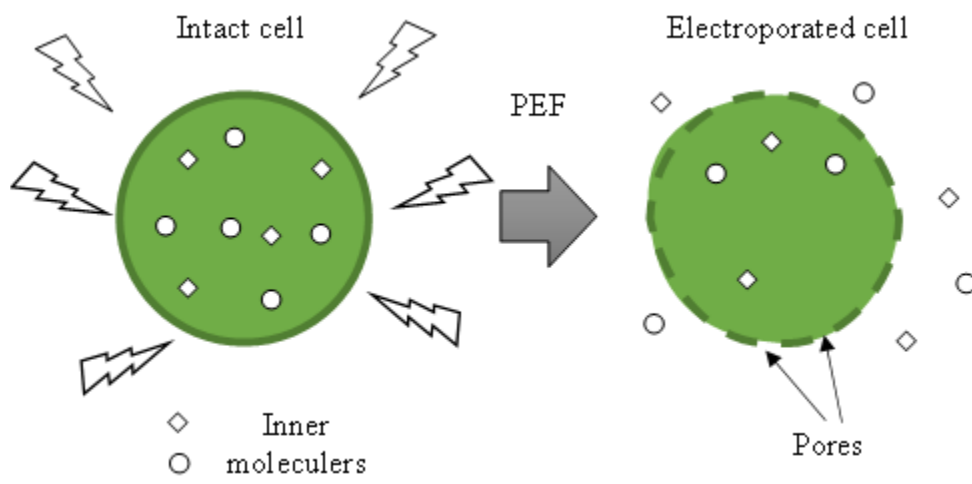


Figure 7