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1 **Effect of combined pulsed electric energy and high pressure**
2 **homogenization on selective and energy efficient extraction of**
3 **bio-molecules from microalga *Parachlorella kessleri***

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19

20 **Abstract**

21 This work investigates the potential of pulsed electric energy (pulsed electric fields
22 (PEF) and high-voltage electrical discharges (HVED)) and high pressure
23 homogenization (HPH) for extraction of bio-molecules from *Parachlorella kessleri*.
24 The applied procedures included individual pulsed electric energy (PEF, HVED), HPH
25 treatments, and their combination. The cell concentrations of suspension applied in PEF
26 or HVED treatment were 5 ~ 25 g/L, while 5 g/L suspension was always applied for
27 HPH treatment. The effects of applied procedures on the extraction of ionic components,
28 carbohydrates, proteins, and pigments were evaluated. The data evidenced that HVED
29 treatment allowed selective extraction of ionic components and carbohydrates, while
30 HPH treatment was most efficient for simultaneous release all the bio-molecules. HPH
31 treatment (120 MPa, 10 passes) gave 4-fold higher content for pigments, 1.2-fold higher
32 content for carbohydrates and 6.5-fold higher content for proteins than HVED treatment
33 (40 kV/cm, 8 ms). For combined procedure, application of preliminary PEF or HVED
34 treatment with 5 g/L suspension allowed increasing the extraction of carbohydrates at
35 high energy consumption. By contrast, application of preliminary HVED treatment with
36 15 g/L or 25 g/L suspensions allowed enhancing extraction efficiencies of carbohydrates
37 and proteins, and reducing total energy consumption.

38 **Keywords:** Pulsed electric fields; High-voltage electrical discharges; Proteins,
39 Carbohydrates, Extraction

40

41 **1. Introduction**

42 Microalgae have been considered as a renewable feedstock for the food, feed and
43 biofuel industries due to their rich composition, superior areal productivities compared
44 to traditional crops and no dependence on fresh water and arable land (Garcia, Safi,
45 Eppink, Wijffels, & Van, 2018). They can also rapidly accumulate biomass and produce
46 desired bio-molecules by change culture growth conditions (like light, nutrient stress
47 and nitrogen starvation, etc) and control their metabolism (Li et al., 2013).

48 The green microalga *Parachlorella kessleri* (*P. kessleri*) is a unicellular freshwater
49 organism (Chlorophyta) with a 2.5-10 μm mean diameter. However, the interesting bio-
50 molecules of microalgae are commonly located either inside the cell cytoplasm or are
51 bound to cell membrane and require disintegration of cell wall before extraction (Garcia
52 et al., 2018). Some efforts have been done to break the microalgal cell wall by chemical
53 hydrolysis (Sedighi et al., 2019), high pressure disruption (Bernaerts, Gheysen, Foubert,
54 Hendrickx, & Van, 2019), ultrasound (Zhang, Grimi, Marchal, Lebovka, & Vorobiev,
55 2019), microwave (Chew, Chia, Lee, Zhu, & Show, 2019), or bead milling (Garcia et al.,
56 2019), etc. However, these technologies refer to the use of severe processing conditions
57 that negatively affect the quality and purity of the extracts and complicating
58 downstream purification processes (Li et al., 2020; Martinez et al., 2019; Martinez,
59 Luengo, Saldana, Alvarez, & Raso, 2017). For example, mechanical disruption was
60 considered as highly energy inefficient, when conducted under laboratory conditions
61 and required a specific energy consumption of at least 33 MJ/kg dry biomass (Lee,

62 Lewis, & Ashman, 2012). These limitations have inspired numerous investigations of
63 alternative methods for recovery of bio-molecules from microalgae.

64 Recently, increasing interest has been focused on the use of pulsed electric fields
65 (PEF) to improve the extraction efficiencies of algal bio-molecules (Jaeschke et al.,
66 2019; Martinez et al., 2019; Parniakov et al., 2015a). PEF treatment can cause the
67 increment of cell membrane permeability (electroporation) by applying high-intensity
68 electric field pulses of short duration (from μ s to ms) (Puertolas & Barba, 2016).
69 However, some research groups have also found that PEF was less effective for
70 extraction of proteins. More efficient extraction of proteins from microalgae required
71 more powerful disintegration of the cell walls, which can be provided by high voltage
72 electrical discharges (HVED) (Grimi et al., 2014). HVED treatment combines electrical
73 and mechanical effects for cell permeabilisation, can cause cell structure damage and
74 accelerate the extraction efficiencies of bio-molecules. Additionally, growing number of
75 researches have been focused on using the combined methods to achieve synergy
76 thereby increasing extraction efficiencies. For example, the use of US in combination
77 with microwave irradiation enhanced oil production from *Chlorella vulgaris* was
78 studied (Ma et al., 2015). Tavanandi, Mittal, Chandrasekhar, & Raghavarao (Tavanandi,
79 Mittal, Chandrasekhar, & Raghavarao, 2018) found that the combined US + freezing
80 and thawing method obtained the highest yield (91.62%) of *C-Phycocyanin* from
81 *Arthospira platensis*, while US (43.05%) or freezing and thawing alone (62.56%).
82 Dejoye et al. (Dejoye et al., 2011) in their work used a combined microwave with

83 supercritical carbon dioxide (SCCO₂) extraction of lipids from *Chlorella vulgaris*,
84 which can be obtained the higher yield of lipid (4.73%) compared to SCCO₂ extraction
85 alone (1.81%).

86 However, to the best of our knowledge, the implementation of energy efficient cell
87 disintegration and selective extraction of microalgal bio-molecules remains largely
88 unexplored. This study investigated the effect of individual pulsed electric energy (PEF,
89 HVED) and HPH treatments, and their combination on the selective extraction of bio-
90 molecules from *P. kessleri*. The dependence of recovery behaviors of ionic components,
91 pigments, carbohydrates and proteins on the different extraction procedures was
92 discussed. The distribution functions of microalgal cells were also observed. Finally, the
93 extraction efficiency in dependence of specific energy consumption and concentration
94 of suspension were evaluated.

95 **2. Materials and methods**

96 *2.1 Chemicals*

97 D-glucose (98%) and bovine serum albumin (BSA, 98%) standard were provided
98 by Sigma-Aldrich (Saint-Quentin Fallavier, France). Bradford Dye Reagent was
99 purchased from Thermo Fisher (Kandel, Germany). Other chemicals of analytical grade
100 were obtained from VWR (Fontenay sous Bois, France).

101 *2.2 Microalgae*

102 Microalga *P. kessleri* was provided by AlgoSolis, Saint-Nazaire, France. The

103 microalga was obtained as frozen paste with moisture of ≈ 820 g H₂O /kg fresh biomass.
104 The major components of biomass include proteins (≈ 440 g/kg dry biomass),
105 carbohydrates (≈ 350 g/kg dry biomass) and lipids (≈ 38 g/kg dry biomass), as well as
106 host of other compounds (chlorophylls, carotenoids, polyphenols and minerals, etc)
107 present in minor quantities. The pastes were first thawed at ambient temperature, and
108 were washed 3 times by deionized water.

109 *2.3 Extraction procedures*

110 The applied extraction procedures included pulsed electric energy (PEF or HVED)
111 treatments, HPH treatment, and pulsed electric energy followed by HPH treatment (i.e.
112 PEF + HPH treatment or HVED + HPH treatment). The untreated (U procedure)
113 suspension was also analyzed as the control experiment.

114 For individual PEF, HVED or HPH treatment, the suspension with the biomass
115 concentration, C_m , of 5 g/L was always used (i.e. $C_m = 5$ g/L). The combined procedure
116 involved using different concentrations of suspension in preliminary pulsed electric
117 energy treatments as follows:

- 118 i) PEF ($C_m = 5$ g/L) + HPH ($C_m = 5$ g/L);
- 119 ii) HVED ($C_m = 5$ g/L) + HPH ($C_m = 5$ g/L);
- 120 iii) HVED ($C_m = 15$ g/L or 25 g/L) + HPH ($C_m = 5$ g/L);

121 For all the combined procedure, a centrifugation step was carried out after pulsed
122 electric energy treatments. Then the residue was re-suspended to 5 g/L suspension by
123 deionized water, and then subjected to HPH treatment.

124 2.3.1. Pulsed electric energy (PEE) treatments

125 The pulsed electric energy treatments were made in PEF and HVED modes using
126 high voltage pulsed power 40 kV-10 kA generator (Basis, Saint-Quentin, France). PEF
127 treatment was performed in a batch one-liter cylindrical treatment chamber between two
128 plate electrodes. The distance between the electrodes was fixed at 2 cm to produce a
129 high PEF intensity of 20 kV/cm. HVED treatment was performed in the same treatment
130 chamber with a needle-plate geometry of electrode. The distance between the stainless
131 steel needle and the grounded plate electrode was fixed at 1 cm and the corresponding
132 electric field strength of 40 kV/cm. The suspension with a total mass of 250 g was
133 introduced between the electrodes. The generator provided pulses of an exponential
134 form with a pulse repetition rate of 0.5 Hz (2 s between pulses: $\Delta t = 2$ s). Treatments
135 comprised application of n successive pulses. The exponential decay of voltage $U \propto$
136 $\exp.(-t/t_p)$ with effective decay time $t_p \approx 10 \pm 0.1 \mu\text{s}$ were observed for applied modes
137 of treatment. The total treatment duration of pulsed electric energy, $t_e = nt_p$, was varied
138 within 0.01–8 ms ($n = 1\text{--}800$). Note that for extraction time of pulsed electric energy
139 was calculated as $t = n \times \Delta t$. Disrupted microalgal suspension characteristics were
140 measured between successive discharges or pulses. The temperature was maintained
141 approximately at ambient temperature, and elevation of temperature not exceeded 5 °C.

142 Specific energy consumption, W (J/kg dry matter), was calculated for pulsed
143 electric energy using the following formula (Yu, Gouyo, Grimi, Bals, & Vorobiev, 2016):

$$W = (n \times P_i)/m \quad (1)$$

144 where m is the mass of the dry weight of biomass in suspension (kg), and P_i refers to the
145 energy consumption of one electric pulse or discharge calculated from the following
146 formula:

$$P_i = (C \times U^2)/2 \quad (2)$$

147 where C is the capacity of the capacitor, and U is the voltage of the generator. In this
148 study, $P_i = 220$ J was applied for all the pulsed electric energy treatments.

149 2.3.2. High pressure homogenization (HPH) treatment

150 The HPH treatment was done using a NS 100L-PANDA 2K two-stage high pressure
151 homogenizer (Niro Soavi S.p.A., Parma, Italy). The average throughput of the
152 homogenizer was 10 L/h. The homogenizing pressure, p , and number of passes, N , were
153 varied within the ranges 40-120 MPa, and 1-10 passes, respectively. The suspensions
154 with a total mass of 250 g were processed. The initial temperature of suspensions before
155 HPH treatment was approximately at ambient temperature and the cooling system to
156 preclude the temperature elevation after HPH treatment never exceeded 5 °C.

157 Specific energy consumption, W (J/kg dry matter), was calculated for HPH
158 treatment using the following formula (Anand, Balasundaram, Pandit, & Harrison,
159 2007):

$$W = pN/C_m\rho \quad (3)$$

160 where ρ ($\approx 10^6$ g/m³) is the density of the suspension.

161 2.4 Characterization

162 2.4.1 Particle size disruption

163 The particle size distribution (PSD) of cells was measured in the range from 0.01 to
164 3000 μm by Malvern Mastersizer 2000 (Orsay, France). The calculated median meter
165 ($d_{(50)}$) was used to monitor the PSD of cells (See, Supplementary materials Fig. S1).

166 2.4.2 Ionic components

167 The electrical conductivity of suspensions was measured with digital
168 Conductivity/TDS meter (Thermo Fisher Scientific, Saint-Herblain, France) at 25°C.

169 2.4.3 Analyses of supernatant

170 The suspensions were centrifuged using a MiniSpin Plus Rotor F-45-12-11
171 (Eppendorf, Le Pecq, France) at 14,100 g for 10 min. The supernatants were collected
172 for characterization analysis.

173 Absorption spectra were measured by a UV/VIS spectrophotometer (Thermo
174 Scientific, Waltham, MA) within 350-900 nm against the blank (with the precision of \pm
175 1 nm). The pigments content was evaluated by analysis of the absorbance of peaks, A , at
176 the wavelength of $\lambda \approx 680$ nm. This peak can be attributed to the absorbance of
177 chlorophylls (Parniakov et al., 2015a).

178 The carbohydrates content, C_c , was measured using a phenol-sulfuric acid method
179 (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The proteins content, C_p , was
180 measured using the method of Bradford (Bradford, 1976). The lipids content was

181 measured using the method of “whole cell analysis” (WCA) (Van Vooren et al., 2012).
182 Briefly, the samples were first mixed with 20 μ L distilled water and 10 μ L butylated
183 hydroxytoluene (20 μ g/ μ L) in clean vials. Then the mixture was suspended in 6 mL of a
184 CHCl_3 : MeOH mixture (2:1, v/v). Vials were maintained for 6 h in the dark under slow
185 agitation. After extraction, the organic and aqueous phases were separated and the
186 solvent of the extracts was evaporated under N_2 flux. 1 mL of CHCl_3 : MeOH mixture
187 (2:1, v/v) was then added and stored at -20°C until analysis. Total fatty acids (TFA)
188 contents in the lipid extracts were quantified by Gas Chromatography-Flame Ionization
189 Detector (GC-FID) (Agilent Technologies Inc., Santa Clara, CA) analysis. Fatty acid
190 methyl ester (FAME) contents in the lipid extracts were quantified after a
191 transesterification step.

192 *2.5 Statistical analysis*

193 Each experiment was repeated at least three times. Data are expressed as mean \pm
194 standard deviation. The error bars, presented on the figures, correspond to the standard
195 deviations.

196 **3. Results and discussion**

197 *3.1 Extraction of bio-molecules*

198 **Fig. 1** presents the changes of relative electrical conductivity, σ/σ_0 , and absorbance
199 of pigments, A , (a), and the contents of carbohydrates, C_c , and proteins, C_p , (b), in the
200 course of individual pulsed electric energy treatments. The data were compared between

201 PEF and HVED treatments. The application of PEF can significantly increase the
202 relative electrical conductivity and the carbohydrates content as compared to the U
203 samples ($t_e = 0$ ms). The maximum values of $\sigma/\sigma_0 \approx 2.2$ and $C_c \approx 40.4$ mg/g were
204 obtained by using the longest electrical treatment duration, $t_e = 8$ ms. However, PEF
205 treatment at $t_e = 8$ ms only gave $A \approx 0.003$, and $C_p \approx 4$ mg/g, respectively. The data
206 evidenced that the PEF treatment was ineffective for extraction of pigments, and less
207 effective for extraction of proteins. In the study of Pataro et al (Pataro et al., 2017), they
208 report that the degree of cell membrane electroporation was not effective enough to
209 release proteins. This possibly reflects that PEF treatment was opening pores on cell
210 membranes, allowing release small-sized cytoplasmic proteins, but most proteins are
211 larger and more bonded to the cell structure (Carullo et al., 2018). Moreover, some other
212 studies claimed to obtain higher amounts of proteins and pigments from
213 *Nannochloropsis* sp., but PEF treatment used in their studies was assisted with organic
214 solvent extraction (Parniakov et al., 2015b, 2015a).

215 In contrast, HVED treatment allowed enhance all measured values as compared to
216 the U samples ($t_e = 0$ ms). The similar extraction behaviors were observed for ionic
217 components, pigments, carbohydrates and proteins. All the measured values increased
218 with the increase of t_e . HVED treatment at $t_e = 8$ ms resulted in the highest values of
219 $\sigma/\sigma_0 \approx 3.1$, $A \approx 0.05$, $C_c \approx 83$ mg/g and $C_p \approx 22$ mg/g. Moreover, note that at $t_e = 6$ ms
220 the measured values were reached the saturated level. Therefore, from the results of **Fig.**
221 **1**, it can be concluded that HVED treatment was more effective than PEF treatment for

222 the recovery of ionic components, pigments, carbohydrates and proteins. However, the
223 spectral data evidenced that pigments extraction was practically absent after both
224 HVED and PEF treatments ($A < 0.1$) since extracted chlorophylls are practically
225 insoluble in water. Their extraction required application of adapted solvents and more
226 intensive cell disruption technologies in the form of pigments-macromolecular water-
227 soluble complexes. This result is in a good agreement with the work of Grimi et al.
228 (Grimi et al., 2014), who studied intracellular components recovery from microalgae
229 *Nannochloropsis* sp.. They find that electrically based disruption techniques were
230 ineffective for extraction of pigments.

231 **Fig. 2** presents the changes of relative electrical conductivity, σ/σ_0 , and absorbance
232 of pigments, A , (a), and the content of carbohydrates, C_c , and proteins, C_p , (b) versus the
233 number of passes, N , for HPH treatment at different applied pressures, $p = 40$ -120 MPa.
234 All the measured values increased with the increase of applied pressure, p , and number
235 of passes, N . The extraction for HPH treatment (120 MPa, $N = 10$) gave the highest
236 values of $\sigma/\sigma_0 \approx 1.9$, $A \approx 0.2$, $C_c \approx 102.6$ mg/g and $C_p \approx 144.2$ mg/g. Note that the
237 application of HPH treatment for $N = 2$ increased the value of σ/σ_0 up to ≈ 1.3 , 1.6, and
238 1.9 for $p = 40$, 80 and 120 MPa, respectively, and no further increase in σ/σ_0 was
239 observed with an increase of N (**Fig. 2a**). However, for pigments, carbohydrates and
240 proteins, the first four passes ($N = 4$) allowed obtaining a noticeable extraction
241 efficiencies for all applied pressures and further passes resulted in insignificant
242 supplementary effects at high specific energy consumption. Therefore, in further

243 combination protocols $N = 4$ for HPH treatment was applied.

244 The obtained data showed that the extraction efficiencies of bio-molecules depend
245 dramatically on the used cell disruption techniques. The pulsed electric energy
246 treatments are more effective for recovery of ionic components than HPH treatment. For
247 both HVED and PEF treatments at $t_e = 8$ ms gave higher value of σ/σ_0 than HPH
248 treatment at 120 MPa for $N = 10$. Note that HVED was also very efficient for the
249 recovery of carbohydrates. HVED treatment at $t_e = 8$ ms gave the similar value of C_c
250 with P procedure for $N = 4$ at 120 MPa. However, HPH treatment can lead to the
251 intensive generation of cell debris and release of all the bio-molecules with increased
252 energy consumption. The application of HPH treatment (120 MPa, $N = 10$) gave 4-fold
253 higher content for pigments and 6.5-fold higher content for proteins as compared to
254 HVED treatment. The application of procedures that combine the important qualities of
255 pulsed electric energy and HPH treatments can be attractive in terms of extraction yield,
256 selectivity and energy consumption.

257 **Fig. 3** compares the contents of carbohydrates, C_c , and proteins, C_p , versus the
258 specific energy consumption, W , for treatment using combined pulsed electric energy
259 and HPH treatments for diluted suspensions with $C_m = 5$ g/L. The treatment duration of
260 pulsed electric energy treatments was $t_e = 2-8$ ms, the applied pressure and number of
261 HPH passes were 80 MPa and $N = 4$, respectively. The used combination procedure
262 consisted in applying $\approx 99-205$ kJ/g dry matter of specific energy consumption. It was
263 observed that HVED + HPH treatment showed higher values when compared to the

264 PEF + HPH treatment at the equivalent energy. The carbohydrates content increased
265 with the energy consumption, the maximum value of $C_c \approx 151.9$ mg/g was observed for
266 HVED ($t_e = 8$ ms) + HPH (80 MPa and $N = 4$) treatment with an energy input of ≈ 204.8
267 kJ/g dry matter. However, the maximum value of $C_p \approx 74.1$ mg/g was observed for
268 HVED ($t_e = 6$ ms) + HPH treatment (≈ 169.6 kJ/g dry matter), further discharges
269 duration in the preliminary HVED treatment resulted in the slight decrease of proteins
270 content in final HPH treatment at high specific energy consumption. This reflects the
271 negative impact of HPH treatment on the microstructure of protein after HVED
272 treatment. A possible explanation for this phenomenon is that the irreversible binding to
273 the cell wall provoked by a longer duration of HVED treatment. However, it still needs
274 to be further proved and identified.

275 Note that for extraction of carbohydrates, the combined treatments can display
276 higher extraction efficiencies. For example, individual pulsed electric energy ($t_e = 4$ ms)
277 or HPH (80 MPa, $N = 4$) treatment for extraction of carbohydrates, and gave $C_c \approx 34.4$
278 mg/g (for PEF treatment), ≈ 66.6 mg/g (for HVED treatment) (**Fig. 1b**), and ≈ 65.2
279 mg/g (for HPH treatment) (**Fig. 2b**). However, the combined pulsed electric energy ($t_e =$
280 4 ms) + HPH (80 MPa, $N = 4$) gave $C_c \approx 104.8$ mg/g (for preliminary PEF treatment)
281 and ≈ 135.2 mg/g (for preliminary HVED treatment) (**Fig. 3**). Similar behaviour was
282 also observed for combined pulsed electric energy ($t_e = 6$ or 8 ms) + HPH (80 MPa, $N =$
283 4) treatments. However, the extraction of proteins for combined method was ineffective
284 when compared with individual HPH treatment (80 MPa, $N = 4$). For example,

285 extraction of proteins using HPH treatment (80 MPa, $N = 4$) gave $C_p \approx 90.4$ mg/g (≈ 64
286 kJ/g dry matter) (**Fig. 2b**), whereas PEF ($t_e = 4$ ms) + HPH (80 MPa, $N = 4$) treatment
287 gave $C_p \approx 56$ mg/g and HVED ($t_e = 4$ ms) + HPH (80 MPa, $N = 4$) treatment gave $C_p \approx$
288 63 mg/g at ≈ 134.4 kJ/g dry matter (**Fig. 3**). Therefore, the application of individual PEF,
289 HVED, HPH or combined treatment should be done based on the most suitable
290 extraction selectivity, purity of extract and energy consumptions.

291 It was observed that the considerable specific energy consumption was needed for
292 diluted suspensions ($C_m = 5$ g/L). For concentrated suspensions, the extraction can be
293 more effective in terms of energy consumption per g dry matter. **Fig. 4** compares
294 extraction behaviours for carbohydrates and proteins after application of combined
295 procedure for concentrated suspensions. In these experiments, 15 g/L or 25 g/L
296 suspensions was used in the preliminary HVED treatment ($t_e = 6$ ms; ≈ 35.2 and 21.2
297 kJ/g dry matter, respectively), and then 5 g/L re-suspensions were used in the final HPH
298 treatment (40-120 MPa, $N = 4$). After the preliminary HVED treatment, the values of C_c
299 ≈ 72.2 mg/g and $C_p \approx 20.6$ mg/g were obtained for 15 g/L suspensions. For 25 g/L
300 suspensions, the values were observed for carbohydrates ($C_c \approx 71.8$ mg/g) and proteins
301 ($C_p \approx 19.0$ mg/g) (**Fig. 4**). Note that for 5 g/L suspensions, the similar preliminary
302 HVED treatment ($t_e = 6$ ms, ≈ 105.6 kJ/g dry matter) gave $C_c \approx 76.6$ mg/g, $C_p \approx 20.4$
303 mg/g (**Fig. 3**). The extracted contents of carbohydrates and proteins were not significant
304 different among different cell concentration of suspensions. This phenomenon was also
305 observed in previously report (Safi et al., 2017), the authors investigated extraction of

306 water-soluble proteins from *Nannochloropsis gaditana* using PEF treatment. They
307 reported the yield of proteins is statistically the same for used concentration of
308 suspension ranging from 15 g/L to 45 g/L.

309 Moreover, the final HPH treatment at 40 MPa (≈ 83.2 kJ/g dry matter) increased the
310 values of C_c up to 113.7 mg/g and C_p up to 42.3 mg/g for 15 g/L suspensions (**Fig. 4**).
311 Similar behaviours were observed for 25 g/L suspensions using lower energy
312 consumption (≈ 69.1 kJ/g dry matter). Furthermore, the values of C_c and C_p
313 continuously increased with the increase of p or E for both 15 g/L and 25 g/L
314 suspensions. For example, at $p = 80$ MPa, they reached the values of $C_c \approx 141.9$ mg/g
315 and $C_p \approx 85.3$ mg/g for 15 g/L suspensions (≈ 131.2 kJ/g dry matter); and the values of
316 $C_c \approx 149.6$ mg/g and $C_p \approx 98.5$ mg/g for 25 g/L suspensions (≈ 117.1 kJ/g dry matter).
317 These values can be compared with the values of $C_c \approx 148.5$ mg/g, and $C_p \approx 74.1$ mg/g,
318 obtained using HVED ($t_e = 6$ ms, $C_m = 5$ g/L) + HPH (80 MPa, $N = 4$) treatment (\approx
319 169.6 kJ/g dry matter) (**Fig. 3**). Therefore, the preliminary HVED treatment of more
320 concentrated suspensions allowed increasing the extraction efficiency and decreasing
321 the energy consumptions.

322 3.2 Selective carbohydrate release

323 For characterization of relative selectivity of carbohydrates and proteins extraction,
324 the selectivity ratio, $S = C_c/C_p$, was used. For non-selective extraction, the value of $S = 1$
325 is expected. S can be regarded as a quality parameter for cell disruption processes, i.e., a
326 higher selectivity for the desired product makes further fraction/purification processing

327 easier (e.g., less impurities) (Postma et al., 2017).

328 **Fig. 5** presents the selectivity ratio, S , versus electrical treatment time, t_e , for
329 individual pulsed electric energy treatments and combined pulsed electric energy ($t_e = 2$ -
330 8 ms) + HPH (80 MPa, 4 passes) treatment for diluted suspensions ($C_m = 5$ g/L) (a), and
331 homogenizing pressure, p , for combined HVED ($t_e = 6$ ms) + HPH (40-120 MPa, $N = 4$)
332 procedure for concentrated suspensions ($C_m = 15$ g/L or 25 g/L) (b). In our case, the
333 applied procedures were selective extraction towards carbohydrates ($S > 1$). The
334 selectivity ratio were smaller with the application of HPH treatment ($S = 1-1.8$) as
335 compared with pulsed electric energy treatments ($S \geq 3.5$). This means that the smallest
336 selectivity was obtained for extraction assisted with HPH treatment. The PEF treatment
337 gave on average the highest selectivity ratio, followed by the HVED treatment.
338 Therefore, for carbohydrates' selective release, the relatively mild cell disruption
339 technique is required.

340 Moreover, for the pulsed electric energy treatments with diluted suspension ($C_m =$
341 5 g/L), the values of S decrease with the increase of t_e (**Fig. 5a**). Note that the
342 application of pulsed electric energy treatments for $t_e = 6$ ms decreased the values to S
343 ≈ 11 and $S \approx 4$ for PEF and HVED treatment, respectively and no further decrease in S
344 was observed with an increase of t_e . This can be explained by a smaller quantity of
345 small-sized water-soluble proteins were released with the longer treatment time,
346 resulted in the decrease of S . However, the selectivity ratio in the final HPH treatment
347 was almost stable ($S = 1-1.5$) regardless of applied t_e in preliminary pulsed electric

348 energy treatments (**Fig. 5a**).

349 Note that for HVED treatment ($t_e = 6$ ms), the similar values were observed ($S \approx 4$)
350 regardless of applied cell concentration (**Fig. 5a and b**). However, the values of S in
351 final HPH treatment applied $p = 40$ MPa ($S = 1.8$) were higher than that obtained from
352 final HPH treatment applied $p = 80$ or 120 MPa ($S = 1$) (**Fig. 5b**). This possibly reflects
353 that applied lower homogenizing pressure was less effective for microalgae cell damage
354 and release larger-size proteins. The finding agree with the previously study (Geciova,
355 Bury, & Jelen, 2002), who reported that pressures of HPH ranging from 55-200 MPa are
356 appropriate for the disruption of microbial cell.

357 Therefore, we can speculate that pulsed electric energy treatments allow selective
358 release small-sized bio-molecules (e.g. ionic components, carbohydrates) resulting in
359 relative pure fractions without negatively harming the other molecules.

360 **4. Conclusion**

361 An ideal extraction technique should be effective on extraction efficiency, energy
362 efficient and scalable. The present study provides an energy efficient method (combined
363 HVED + HPH treatment) for obtaining intracellular bio-molecules (carbohydrates and
364 proteins) of *P. kessleri* with high extraction efficiency. The extraction efficiency and
365 energy consumption depends on the cell concentration of prior HVED treatment. The
366 increase of concentration from 5 g/L to 25 g/L resulted in ≈ 5 -fold decrease of HVED
367 energy consumption at approximately the same efficiency of extraction of carbohydrates
368 and proteins. Furthermore, HVED pre-treated more concentrated suspension (15 g/L or

369 25 g/L) underwent HPH allowed enhancing extraction efficiencies of proteins and
370 reducing total energy consumption, compared to HVED pre-treated diluted suspension
371 (5 g/L) followed by HPH. This promising method results can improve the recovery of
372 microalgae intracellular bio-molecules avoiding the use of solvents, reducing the energy
373 consumption of HPH.

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378

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510

Figure captions

Fig. 1 Relative electrical conductivity, σ/σ_0 , after and before treatment, and absorbance of pigments, A , at the wavelength of $\lambda = 680$ nm (a), and the contents of carbohydrates, C_c , and proteins, C_p , (b), versus PEE treatment time, t . The suspension's concentration was $C_m = 5$ g/L. (PEE: pulsed electric energy)

(—▲— : HVED; —■— :PEF)

Fig. 2 Relative electrical conductivity, σ/σ_0 , after and before treatment, and absorbance of pigments, A , at the wavelength of $\lambda = 680$ nm (a), and the contents of carbohydrates, C_c , and proteins, C_p , (b), versus the number of HPH passes, N , at different pressures (40–120 MPa). The suspension's concentration was $C_m = 5$ g/L dry matter. (HPH: high pressure homogenization)

(—▲— :40 Mpa; —▶— :80 Mpa; —◆— : 120 Mpa)

Fig. 3 The contents of carbohydrates, C_c , and proteins, C_p , versus the specific energy consumption, W , for combined PEE (PEF or HVED; 2-8ms) and HPH (80 MPa, 4 passes) treatment. The suspension's concentration was $C_m = 5$ g/L dry matter. (PEE: pulsed electric energy; HVED: high-voltage electrical discharges; PEF: pulsed electric fields; HPH: high pressure homogenization)

(—■— : PEF; —□— : PEF+HPH; —▲— : HVED; —△— :HVED+HPH)

Fig. 4 The content of carbohydrates, C_c , and proteins, C_p , versus the specific energy consumption, W , for combined HVED (15 or 25 g/L, 6 ms) and HPH (5 g/L, 40-120 MPa, 4 passes) treatment. (HVED: high-voltage electrical discharges; HPH: high

532 pressure homogenization)

533 (■: HVED (6 ms, 15 g/L) ; ▲: HVED (6 ms, 15 g/L)+HPH (40 MPa, 5 g/L);

534 ►: HVED (6 ms, 15 g/L)+HPH (80 MPa, 5 g/L); ◆: HVED (6 ms, 15 g/L)+HPH (120

535 MPa, 5 g/L);

536 □: HVED (6 ms, 25 g/L) ; △: HVED (6 ms, 25 g/L)+HPH (40 MPa, 5 g/L);

537 ▷: HVED (6 ms, 25 g/L)+HPH (80 MPa, 5 g/L);◇: HVED (6 ms, 25 g/L)+HPH (120

538 MPa, 5 g/L))

539 **Fig. 5** The selectivity ratio, S , versus electrical treatment time, t_e , for individual PEE

540 (PEF or HVED; 5 g/L, 2-8 ms) and combined PEE (PEF or HVED; 5 g/L, 2-8 ms) and

541 HPH (80 MPa, 4 passes) treatment (a), and versus homogenizing pressure, p , for

542 combined HVED (15 g/L or 25 g/L, 6 ms) + HPH (5 g/L, 40-120 MPa, 4 passes)

543 treatment (b). (PEE: pulsed electric energy; HVED: high-voltage electrical discharges;

544 PEF: pulsed electric fields; HPH: high pressure homogenization)

545 (a: ▲ : PEF; △ : PEF+HPH; ■ : HVED; □ :HVED+HPH;

546 b: ▲ :15 g/L; ◆ : 25 g/L)

547

a)

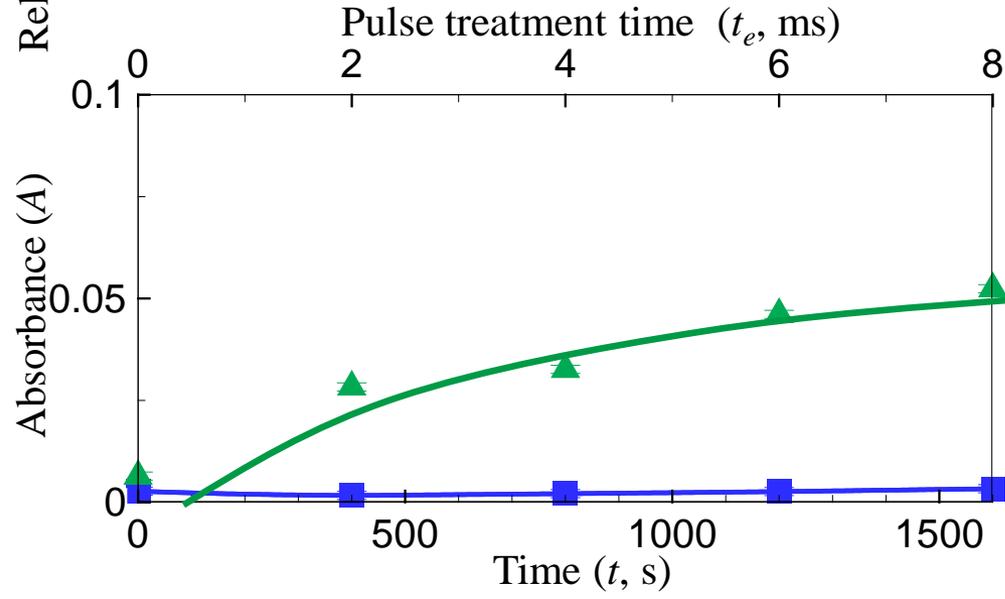
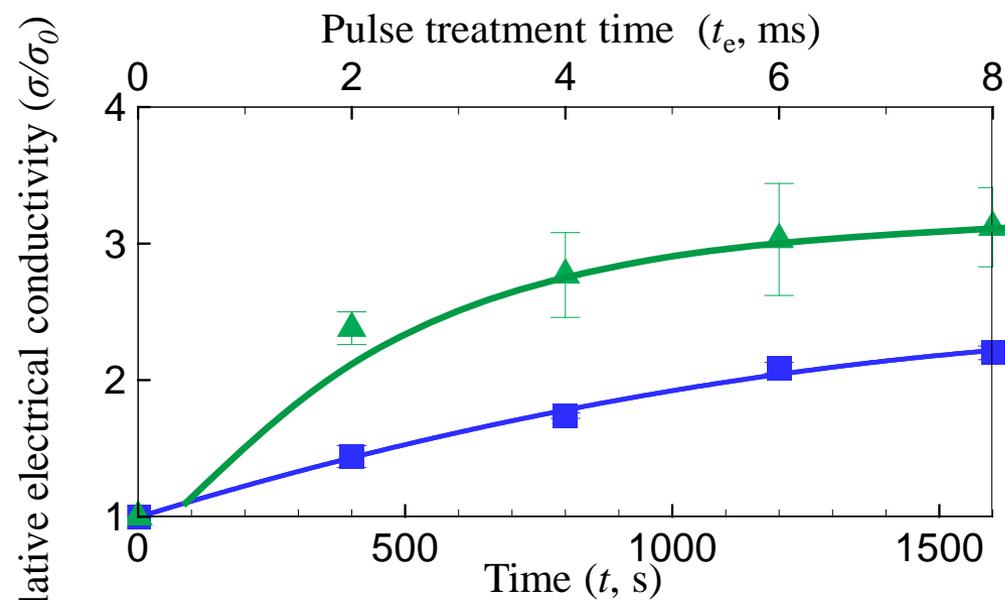


Fig. 1a

b)

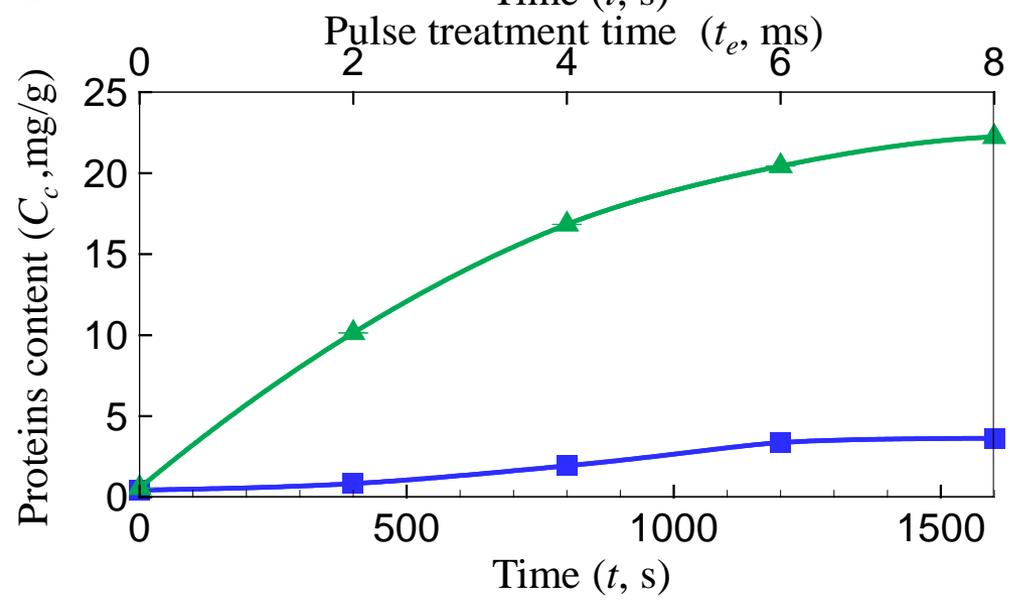
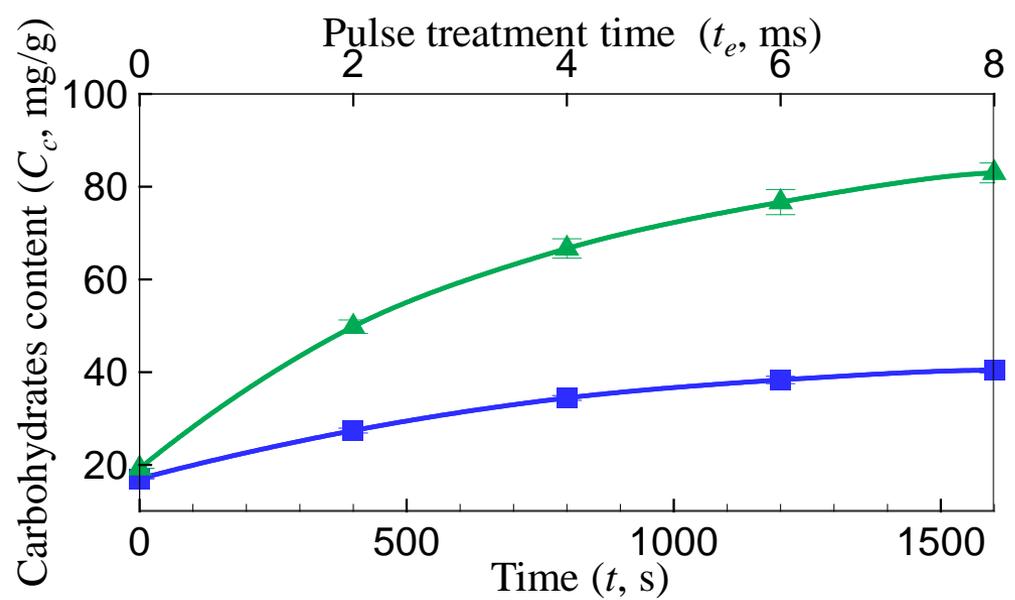


Fig. 1b

a)

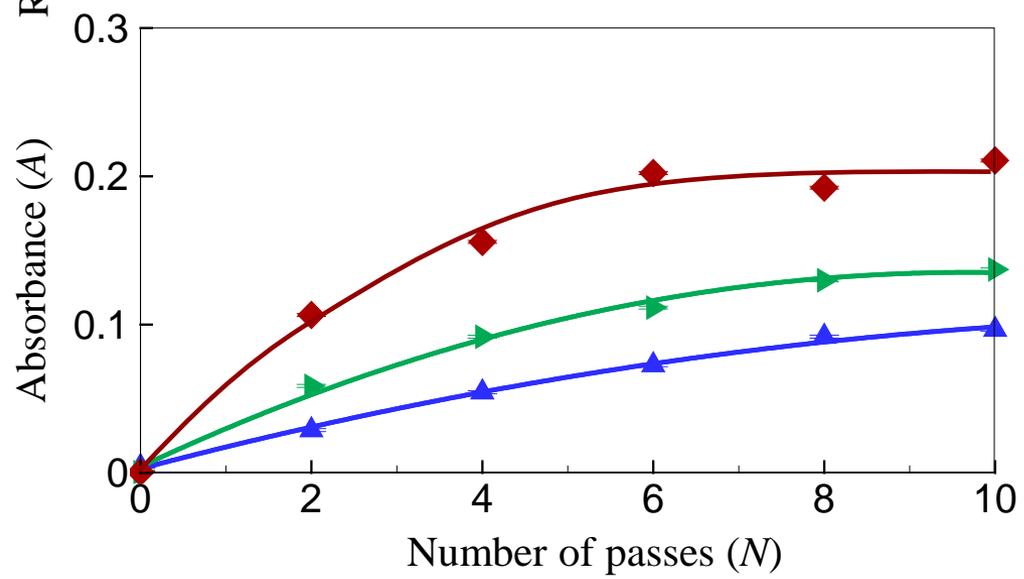
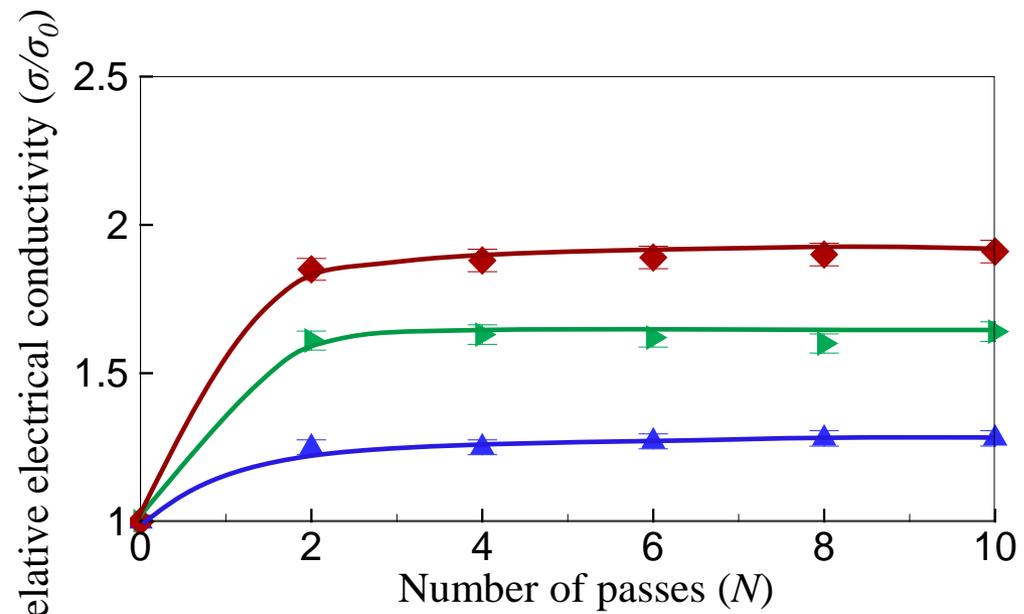


Fig. 2a

b)

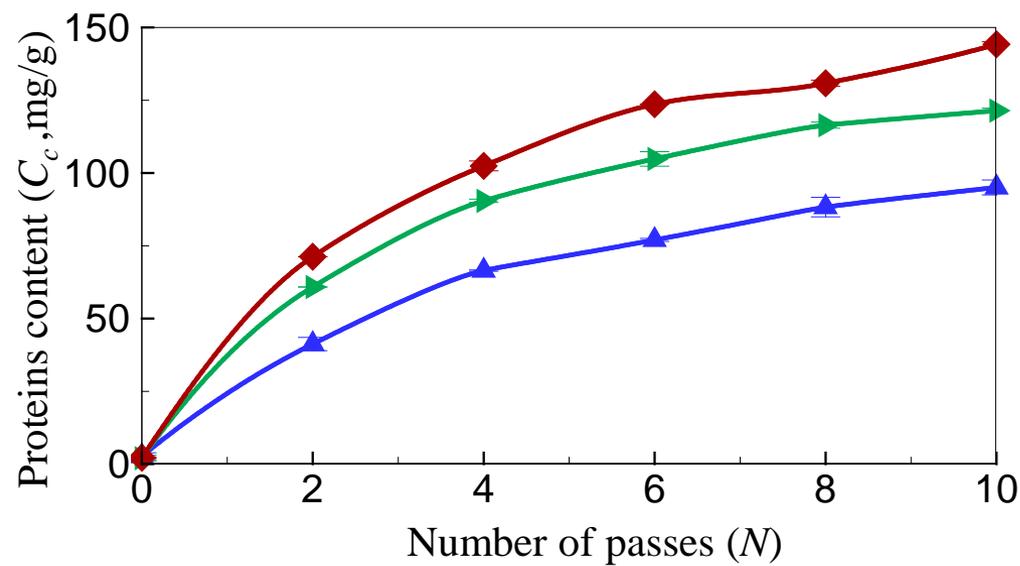
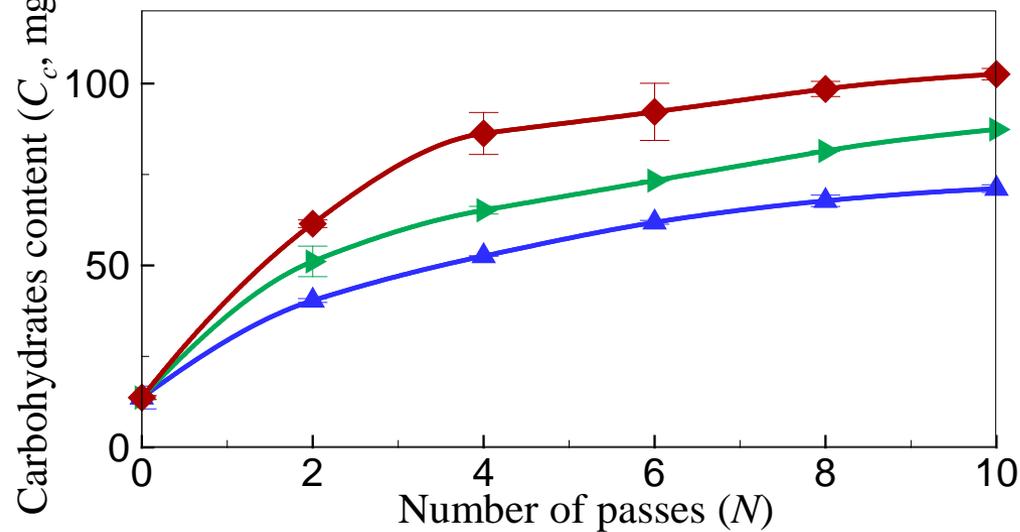


Fig. 2b

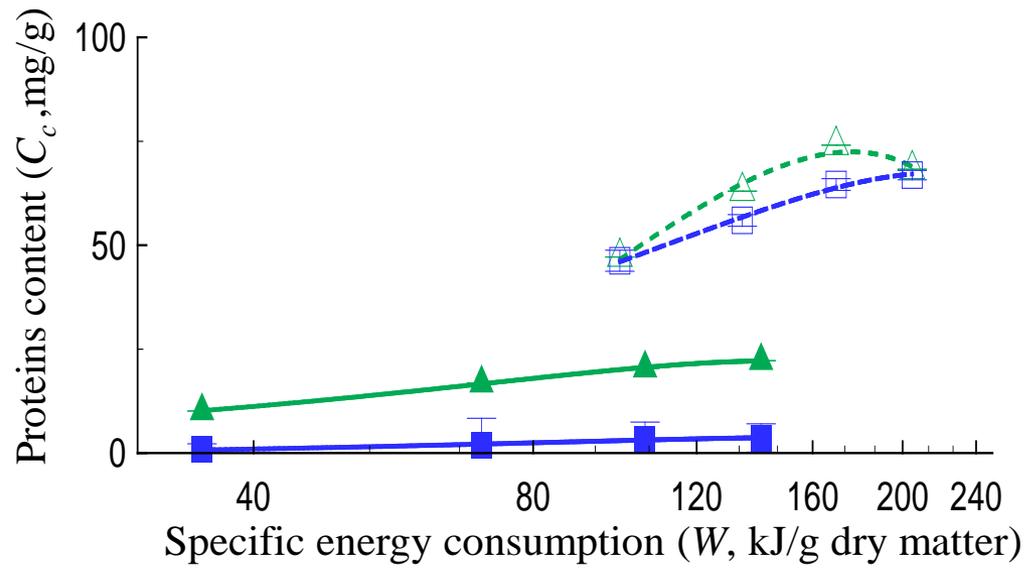
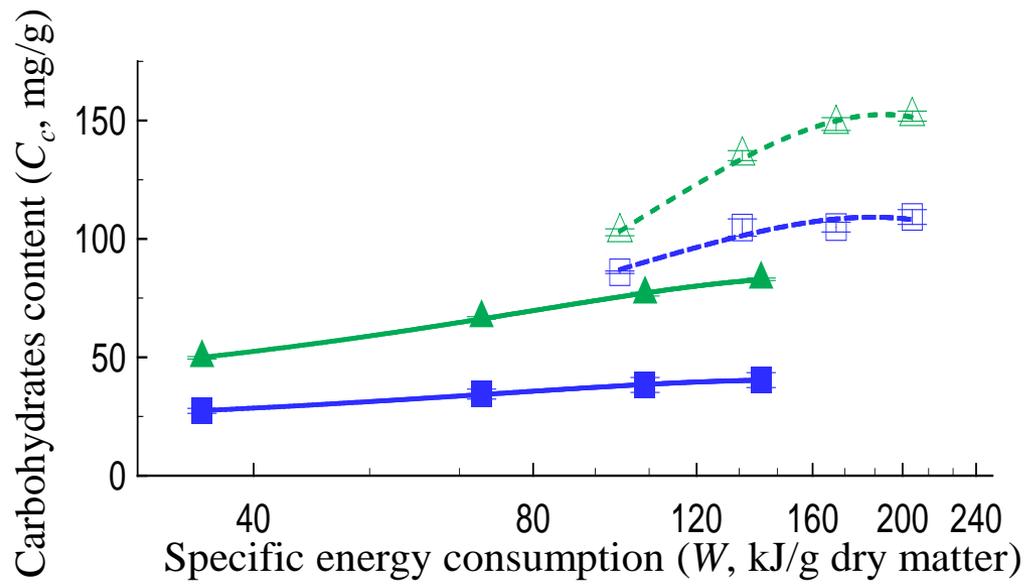


Fig. 3

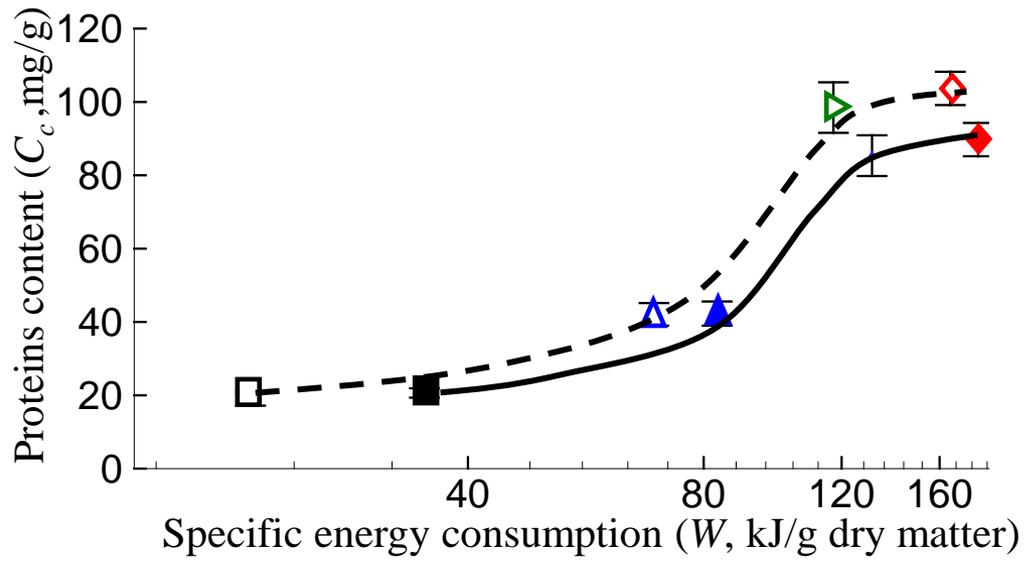
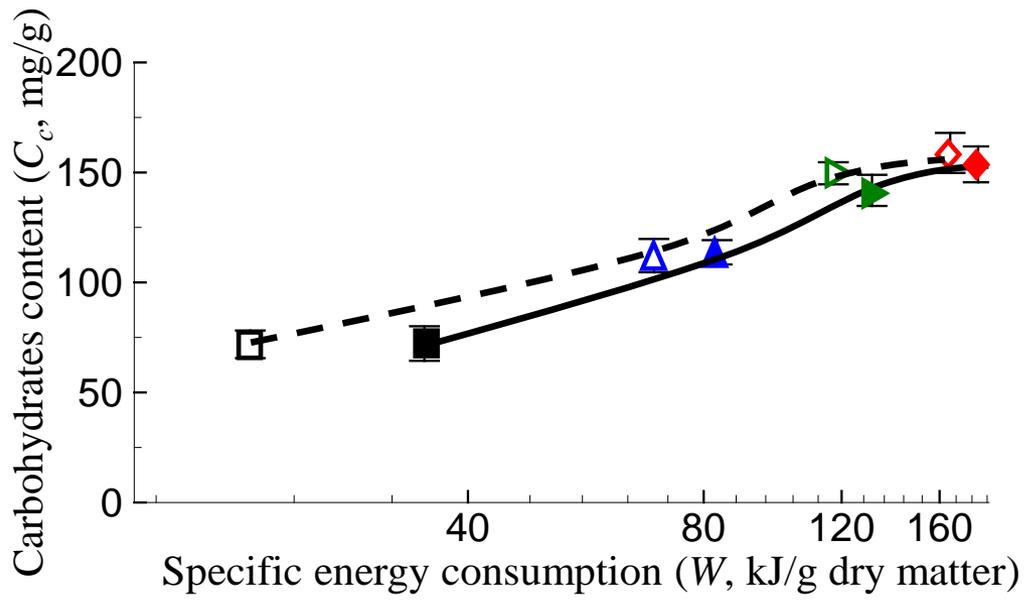


Fig. 4

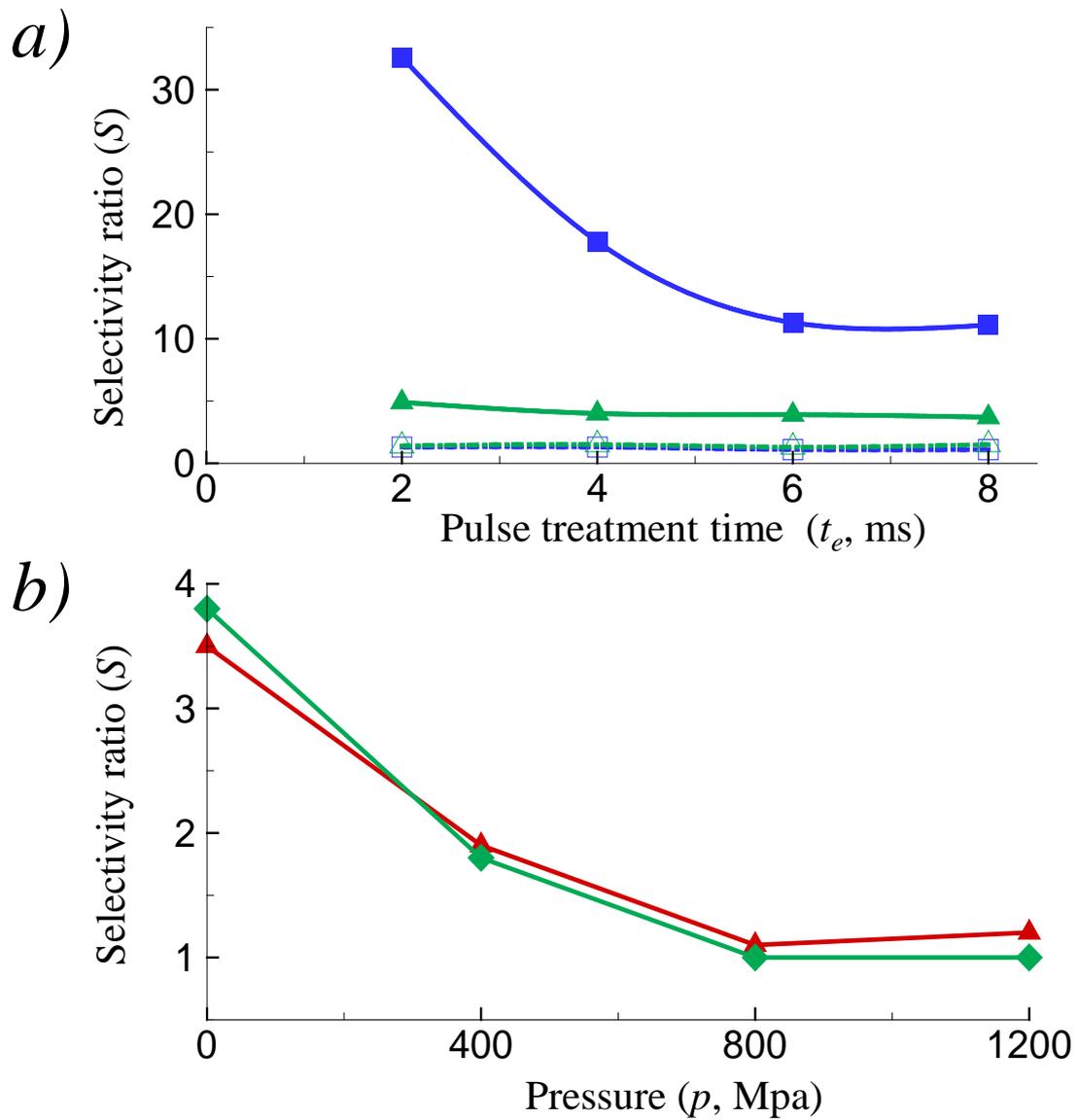


Fig. 5

Untreated (U) microalgal suspensions
($C_m=5\text{g/L}$)

Individual PEE procedure ($C_m=5\text{g/L}$):

PEF treatment (20 kV/cm; 1-8 ms);
HVED treatment (40 kV/cm; 1-8 ms)

Individual HPH procedure ($C_m=5\text{g/L}$):

HPH treatment:
(40, 80, 120 MPa; 1-10 passes)

PEE ($C_m=5\text{g/L}$) + HPH ($C_m=5\text{g/L}$):

PEF (2,4,6,8 ms)+HPH (80 MPa, 4 passes);
HVED (2,4,6,8 ms)+HPH (80 MPa, 4 passes)

PEE ($C_m=15$ or 25g/L) + HPH ($C_m=5\text{g/L}$):

HVED (6 ms)+HPH
(40, 80, 120 MPa; 4 passes)

Characterization:

Suspension : ionic components; size distribution
Supernatant : proteins; carbohydrates; pigments