



**HAL**  
open science

# Pulsed electric energy and ultrasonication assisted green solvent extraction of bio-molecules from different microalgal species

Rui Zhang, Nikolai Lebovka, Luc Marchal, Eugène Vorobiev, Nabil Grimi

## ► To cite this version:

Rui Zhang, Nikolai Lebovka, Luc Marchal, Eugène Vorobiev, Nabil Grimi. Pulsed electric energy and ultrasonication assisted green solvent extraction of bio-molecules from different microalgal species. *Innovative Food Science & Emerging Technologies / Innovative Food Science and Emerging Technologies*, 2020, 62, pp.102358 -. 10.1016/j.ifset.2020.102358 . hal-03490521

**HAL Id: hal-03490521**

**<https://hal.science/hal-03490521v1>**

Submitted on 22 Aug 2022

**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.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1    **Pulsed electric energy and ultrasonication assisted green solvent**  
2    **extraction of bio-molecules from different microalgal species**

3    Rui Zhang<sup>1</sup>, Nikolai Lebovka<sup>1,2</sup>, Luc Marchal<sup>3</sup>, Eugène Vorobiev<sup>1</sup>, Nabil Grimi<sup>1\*</sup>

4

5    <sup>1</sup>Sorbonne University, Université de Technologie de Compiègne, ESCOM, EA 4297 TIMR, Centre de  
6    recherche Royallieu - CS 60319 - 60203 Compiègne cedex, France

7    <sup>2</sup>Institute of Biocolloidal Chemistry named after F. D. Ovcharenko, NAS of Ukraine, 42, blvr.  
8    Vernadskogo, Kyiv 03142, Ukraine

9    <sup>3</sup>LUNAM Université, CNRS, GEPEA, Université de Nantes, UMR6144, CRTT, Boulevard de  
10   l'Université, BP 406, 44602 Saint-Nazaire Cedex, France

11

12

13   \*Corresponding Author Address:

14   Dr. Nabil Grimi

15   Sorbonne University, Université de Technologie de Compiègne, ESCOM, EA 4297 TIMR, Centre de  
16   recherche Royallieu - CS 60319 - 60203 Compiègne cedex, France

17   E-mail address: [nabil.grimi@utc.fr](mailto:nabil.grimi@utc.fr)

18

19 **Abstract**

20 The effects of physical treatments (pulsed electrical fields (PEF), high voltage electrical discharges  
21 (HVED) and ultrasonication (US)) on aqueous extraction of carbohydrates and proteins, and ethanolic  
22 extraction of chlorophyll *a* from three microalgal species (*Nannochloropsis* sp., *P. tricornutum* and *P.*  
23 *kessleri*) have been studied. The total energy consumption of 530 kJ/kg suspension was applied for each  
24 treatment. For studied species, HVED was the most effective for extraction of carbohydrates, while US  
25 was the most effective for extraction of proteins and chlorophyll *a*. The observed differences for studied  
26 species can reflect the more fragile cell wall structure for *P. tricornutum* as compared with  
27 *Nannochloropsis* sp. or *P. kessleri*. The applied PEE of US treatments along with combinations of  
28 aqueous extraction of carbohydrates and proteins, and ethanolic extraction of pigments can be used in  
29 future implementations of selective extraction of valuable bio-molecules from microalgae.

30 **Keywords:** Carbohydrates; Microalgae; Pigments; Proteins; Pulsed electric energy; Ultrasonication

31

32 **1. Introduction**

33 Microalgae are microscopic unicellular organisms capable to covert solar energy to chemical energy  
34 via photosynthesis (Hosikian, Lim, Halim, & Danquah, 2010) and they have the advantage of capturing  
35 CO<sub>2</sub> from the environment and combustion processes, thereby reducing greenhouse gases (Gerde,  
36 Montalbo-Lomboy, Yao, Grewell, & Wang, 2012). Microalgae can accumulate large amounts of  
37 metabolites over short periods of time, including carbohydrates, proteins, and lipids, as well as pigments  
38 (Khili, 2013; Sankaran et al., 2018). For example, for cultivation of *Chlorella vulgaris* under illumination  
39 with red light, the highest yield of chlorophyll *a* was achieved, corresponding to 1.29% of cell biomass  
40 (da Silva Ferreira & Sant' Anna, 2017). The chlorophyll content can be also greatly affected by the  
41 cultivation temperature, stirring the microalgal culture and content of nutrients (nitrogen, phosphorus) and  
42 micronutrients (iron, zinc, manganese, copper)

43 Natural pigments have an important role in the photosynthetic metabolism and pigmentation of  
44 microalgae (D'Alessandro & Antoniosi Filho, 2016). The major photosynthetic pigments are represented  
45 by chlorophylls, violaxanthin, and vaucheraxanthin in microalgae (Reboloso-Fuentes, Navarro-Pérez,  
46 Garcia-Camacho, Ramos-Miras, & Guil-Guerrero, 2001). Commonly, there exists a directly proportional  
47 relationship between content of chlorophyll *a* and the amount of algal biomass (Henriques, Silva, &  
48 Rocha, 2007).

49 However, the extraction of valuable bio-molecules from the microalgae is not an easy task. These  
50 bio-molecules are commonly located in the intracellular compartments, protected by the rigid cell walls,  
51 and membranes surrounding the cytoplasm and the internal organelles (Postma et al., 2017). For example,  
52 the proteins are commonly located in the cell walls, cytoplasm and chloroplasts, whereas the pigments

53 (chlorophylls and some carotenoids) are located in the thylakoids of the chloroplasts. Different techniques  
54 to release water-soluble bio-molecules from microalgae by chemical hydrolysis (Duongbia, Chaiwongsar,  
55 Chaichana, & Chaiklangmuang, 2019; Lorenzo-Hernando, Ruiz-Vegas, Vega-Alegre, & Bolado-  
56 Rodriguez, 2019; Sedighi, Jalili, Darvish, Sadeghi, & Ranaei-Siadat, 2019), bead milling (Garcia, Lo,  
57 Eppink, Wijffels, & van den Berg, 2019; Rivera et al., 2018), high pressure homogenization (Pataro et al.,  
58 2017), ultrasonication (US) (Gonzalez-Balderas, Velasquez-Orta, Valdez-Vazquez, & Ledesma, 2020;  
59 Skorupskaite, Makareviciene, Sendzikiene, & Gumbyte, 2019; Zhang, Grimi, Marchal, Lebovka, &  
60 Vorobiev, 2019), pulsed electrical fields (PEF) (Parniakov et al., 2015b, 2015a; Pataro et al., 2017), high  
61 voltage electrical discharges (HVED) (Grimi et al., 2014) have been applied. Note that pigments are  
62 poorly soluble in water (practically insoluble), the classical organic solvent extraction or supercritical  
63 fluid extraction are commonly used for their extraction (Hosikian et al., 2010; Macias-Sánchez et al.,  
64 2008). However, as far as we know the PEF and HVED techniques were still rarely applied for assistance  
65 of extraction of insoluble pigments.

66 The main aim of this work is to explore the feasibility of physical treatments (PEF, HVED and US)  
67 to recovery of water-soluble (proteins and carbohydrates) and -insoluble (chlorophyll *a*) bio-molecules  
68 from different microalgal species. The data were compared for three different microalgal species  
69 (*Nannochloropsis* sp., *Phaeodactylum tricornutum* (*P. tricornutum*), and *Parachlorella kessleri* (*P.*  
70 *kessleri*)). These species have different cell shapes, structures and biomass composition. The cells of  
71 *Nannochloropsis* sp. (marina green algae), and *P. kessleri* (freshwater green algae) are approximately  
72 spherical (2–4 µm in diameter), while the cells of *P. tricornutum* (marina diatom) have the fusiform shape  
73 (similar to the lemon-shape) with a length of 20–30 µm and a diameter of 1-3 µm (Alhattab,

74 Kermanshahi-Pour, & Brooks, 2019). Besides, *Nannochloropsis* sp. and *P. kessleri* cells have strong and  
75 rigid cell walls mainly composed of cellulose and hemicelluloses (Payne & Rippingale, 2000), while the  
76 cell walls of *P. tricornutum* are poor in silica and composed of sulfated glucomannan (Francius, Tesson,  
77 Dague, Martin-Jézéquel, & Dufrêne, 2008). The studied microalgal species have different contents of  
78 carbohydrates, proteins, total pigments and lipids. For example, the *Nannochloropsis* sp. contains  
79 maximum content of lipids ( $\approx 9.0\%$  (wt. DW biomass) as compared with *P. tricornutum* ( $\approx 3.9\%$ ) and *P.*  
80 *kessleri* ( $\approx 3.8\%$ ), whereas the *P. kessleri* contains maximum contents of carbohydrates ( $\approx 35\%$ ) as  
81 compared with *Nannochloropsis* sp. ( $\approx 11\%$ ) and *P. tricornutum* ( $\approx 12.4\%$ ). The impact of different  
82 physical treatments on bio-molecules extractability was discussed at equivalent energy consumption.  
83 Attention was also focused on the effects of physical treatments on extraction kinetics of chlorophyll *a*.

## 84 **2. Materials and methods**

### 85 *2.1 Chemicals*

86 D-glucose, bovine serum albumin (BSA) and chlorophyll *a* standard ((#C5753) were provided from  
87 Sigma-Aldrich (Saint-Quentin Fallavier, France). Bradford Dye Reagent and ethanol (EtOH, 95%, v/v)  
88 were obtained from Thermo Fisher (Kandel, Germany). Sulfuric acid and phenol were purchased from  
89 VWR (France).

### 90 *2.2 Microalgae*

91 Three microalgal species (*Nannochloropsis* sp., *P. tricornutum* and *P. kessleri*) were provided by  
92 AlgoSolis (Saint-Nazaire, France). The details of cultivation procedures were described previously for  
93 *Nannochloropsis* sp. (Parniakov et al., 2015a), *P. tricornutum* (Guihéneuf et al., 2011) and *P. kessleri*

94 (Zhang et al., 2019).

95 The samples were obtained as frozen microalgal pastes with  $\approx 80\%$  (*Nannochloropsis* sp.),  $\approx 70\%$  (*P.*  
96 *tricornutum*) and  $\approx 82\%$  (*P. kessleri*) of moisture content, respectively. The pastes were preliminary  
97 washed 3 times using deionized water. Briefly, in the applied washing procedure, the biomass was diluted  
98 to 1% dry matter (DM) (hereinafter %), agitated at 150 rpm for 10 min, and centrifuged for 10 min at  
99 4600 g. Then supernatant was removed, and the sediment was freeze-dried using a MUT 002A pilot  
100 freeze-drier (Cryotec, France) for 64 h at  $-20\text{ }^{\circ}\text{C}$ . The composition of the biomass was determined  
101 according to the previously described methods (Macias-Sánchez et al., 2008; Phelippe, Goncalves,  
102 Thouand, Cogne, & Laroche, 2019; Ritchie, 2006). The carbohydrate content was determined after two  
103 passes in high pressure disrupter (CellD, Constant System) at 270 MPa and centrifugation at 3,000g for 5  
104 minutes. The protein concentration was determined in the total high-pressure lysate. The total pigment  
105 content was measured after centrifugation of intact cells and methanol extraction. The analysis' data gave  
106 that *Nannochloropsis* sp. contains  $\approx 11\%$  (wt. DW biomass) of carbohydrates,  $\approx 43.7\%$  of proteins,  $\approx$   
107  $2.0\%$  of total pigments and  $\approx 9.0\%$  of lipids; *P. tricornutum* contains  $\approx 12.4\%$  of carbohydrates,  $\approx 28.7\%$   
108 of proteins,  $\approx 1.0\%$  of total pigments and  $\approx 3.9\%$  of lipids; *P. kessleri* contains  $\approx 35\%$  of carbohydrates,  $\approx$   
109  $44\%$  of proteins,  $\approx 4.0\%$  of total pigments and  $\approx 3.8\%$  of lipids.

### 110 2.3 Extraction procedures

111 **Fig. 1** presents the schema of the applied experimental procedures. The applied procedure include  
112 cell disintegration by physical treatments (leaching in pure water), and common extraction using green  
113 solvent for recovery of chlorophyll *a*. In control experiments, untreated (U) suspensions were also  
114 analyzed.

115 2.3.1 Physical treatments

116 For physical treatments, the suspensions with the biomass concentrations of 1% were prepared. All  
117 treatments (PEF, HVED and US) were performed using 250 g of the suspension.

118 Treatments by pulsed electric energy (PEF or HVED) were done using a high voltage pulsed power  
119 generator (40 kV-10 kA, Basis, Saint-Quentin, France) in cylindrical batch treatment chamber with  
120 different types of electrodes (**Fig. 2a**). The PEF treatment was done between two parallel plate electrodes.  
121 The distance between the electrodes was fixed at 2 cm to produce the electric field strength of  $E = 20$   
122 kV/cm. The HVED treatment was performed using electrodes in needle-plate geometry. The distance  
123 between the stainless steel needle and the grounded plate electrodes was fixed at 1 cm, the corresponding  
124 electric field strength of  $E = 40$  kV/cm. The protocols of pulsed electric energy (PEF or HVED)  
125 treatments comprised application of  $n = 600$  successive pulses with a frequency of 0.5 Hz, the total  
126 electrical treatment duration was  $t_e = 0.01$ -6 ms. The exponential decay of voltage  $U \propto \exp(-t/t_p)$  with  
127 effective decay time  $t_p \approx 10 \pm 0.1 \mu\text{s}$  were observed for applied modes of treatment (**Fig. 2b**).

128 Specific energy consumption,  $W$  (J/kg suspension), was calculated for PEF and HVED treatment as  
129 following formula (Yu, Gouyo, Grimi, Bals, & Vorobiev, 2016):

$$W = (C \times U^2 \times n) / 2m \quad (1)$$

130 where  $C$  is the capacitance of the capacitor;  $U$  is the voltage of the generator;  $n$  is number of pulses;  $m$  is  
131 the mass of 1% suspension (kg).

132 Treatment by US, a UP-400S ultrasound processor (Hielscher GmbH, Germany) with a constant  
133 frequency of 24 kHz was applied. The ultrasound probe (diameter: 14 mm, length: 100 mm) was plunged  
134 into a beaker, containing of suspension. The total treatment time of US was  $t_u = 660$  s, and the amplitude  
135 was fixed at 50%, which corresponded to the power,  $W_u = 200$  W.

136 Specific energy consumption,  $W$  (J/kg suspension), was calculated for US treatment as following

137 formula:

$$E = W_u \times t_u / m \quad (2)$$

138 where  $m$  is the mass of 1% suspension (kg).

139 For all treatments (PEF, HVED and US), the samples were cooled in order to prevent overheating,  
140 the temperature was maintained approximately at ambient temperature, and elevation of temperature not  
141 exceeded 5 °C.

### 142 2.3.2 Solvent extraction

143 After physical treatments, the microalgal cells were dried by vacuum in a vacuum chamber (Cole-  
144 Parmer, USA) at pressure of 30 kPa, and temperature of 50 °C for 24 h. Then dried biomass was mixed  
145 with EtOH (95%, v/v) with solid-liquid ratio of 1: 20 (w/w) and solvent extraction under the stirring at  
146 150 rpm was done for the time of  $t = 1440$  min (24 h). To avoid any evaporation, the extraction cells were  
147 covered with aluminum foil during the solvent extraction process. The pigments content was measured  
148 continuously during extraction.

### 149 *2.4 Characterization*

150 The supernatant was collected by centrifugation using a MiniSpin Plus Rotor F-45-12-11  
151 (Eppendorf, France) at 14,100 g for 10 min, and then used for analysis. All the characterization  
152 measurements were done at ambient temperature.

#### 153 2.4.1 Carbohydrates analysis

154 The carbohydrates content,  $C_c$ , was determined by phenol-sulfuric acid method (Dubois, Gilles,

155 Hamilton, Rebers, & Smith, 1956). D-glucose was used as a standard. Briefly, 1 mL of supernatant was  
156 mixed with 0.1 mL of phenol solution (5%, w/w) and 5 mL of concentrated sulfuric acid. The mixture  
157 was stored at 20 °C for 20 min. The absorbance was measured at the wavelength  $\lambda = 490$  nm using a  
158 UV/VIS spectrophotometer Spectronic Genesys 20 (Thermo Electron Corporation, MA). The extraction  
159 yield of carbohydrates,  $Y_c$  (%), was calculated by as following formula:

$$Y_c = C_c / C_c^{max} \times 100 \quad (3)$$

160 where  $C_c^{max}$  the total carbohydrate content in microalgae.

#### 161 2.4.2 Proteins analysis

162 The proteins content,  $C_p$ , was determined using the method of Bradford (Bradford, 1976). BSA was  
163 used as a standard. Briefly, 0.1 mL of supernatant was mixed with 1 mL of Bradford Dye Reagent. The  
164 mixture was vortex for 10 s and kept for 5 min. The absorbance was measured at the wavelength  $\lambda = 595$   
165 nm using a UV/VIS spectrophotometer Spectronic Genesys 20 (Thermo Electron Corporation, MA). The  
166 extraction yield of proteins,  $Y_p$  (%), was calculated by as following formula:

$$Y_p = C_p / C_p^{max} \times 100 \quad (4)$$

167 where  $C_p^{max}$  the total proteins content in microalgae.

#### 168 2.4.3 Pigments analysis

169 Absorption spectra of supernatants (diluted if required) obtained from solvent extraction procedure  
170 was measured in the wavelength range of 300-900 nm against the blank (with the precision of  $\pm 1$  nm)  
171 (See, Supplementary materials Fig. S1). The maximum absorbance of chlorophyll *a* of all microalgal  
172 species was at the wavelength of  $\lambda = 660$  nm. The content of chlorophyll *a*,  $C_{chl a}$ , in the extracts were  
173 estimated by chlorophyll *a* calibration curve ( $A=87.86 \times C + 0.0055$ ,  $R^2=0.9998$ ).

174 2.5 Statistical analysis

175 Each experiment was repeated at least three times. Data are expressed as mean  $\pm$  standard deviation.

176 The error bars, presented on the figures, correspond to the standard deviations.

177 **3. Results and discussion**

178 3.1 Extractability of carbohydrates and proteins

179 The cell disintegration efficiency of tested physical treatments at equivalent energy consumption  
180 were evaluated by monitoring the extractability of water-soluble carbohydrates (small-size molecules)  
181 and proteins (large-size molecules), respectively. **Fig. 3** presents the extraction yields of carbohydrates,  $Y_c$ ,  
182 and proteins,  $Y_p$ , in the supernatants, obtained from three microalgal species after application of different  
183 physical treatments. The total energy consumption for all treatments was the same,  $W \approx 530$  kJ/kg  
184 suspension. At this energy consumption, the pulsed electric energy treatments were sufficient for  
185 extraction of water-soluble bio-molecules (e.g. proteins) and further increase in  $W$  resulted in insignificant  
186 supplementary effects (Grimi et al., 2014; Parniakov et al., 2015b).

187 The lowest extraction yields of carbohydrates ( $Y_c = 4\text{-}11.5\%$ ) and proteins ( $Y_p = 0.1\text{-}3.5\%$ ) were  
188 obtained for U samples for each tested specie. The application of physical treatments improved the  
189 extraction yields for both carbohydrates and proteins, as compared to the U samples. The higher  
190 extraction yields for carbohydrates as compare to that for proteins were observed. The highest extraction  
191 yield of carbohydrates ( $Y_c \approx 37.5 \pm 1.8 \%$  using the HVED treatment) was obtained for *Nannochloropsis*  
192 sp., while the highest extraction yield of proteins ( $Y_p \approx 10.1 \pm 0.3 \%$  using the US treatment) was obtained  
193 for *P. tricornutum*. For all tested species, the extraction yields of carbohydrates and proteins can be

194 arranged in the rows of U < PEF < US < HVED and U < PEF < HVED < US, respectively. Therefore, the  
195 HVED treatment was the most efficient technique to extract carbohydrates, while US treatment was the  
196 most efficient technique to extract proteins. However, at once the PEF treatment obtained the smallest  
197 extraction efficiencies of water-soluble bio-molecules for all tested species. This reflects that the  
198 electroporation mechanism itself was not very effective for extraction of intracellular molecules as  
199 compared with efficiency of HVED and US treatments (Pataro et al., 2017). The observed data are in  
200 agreement with previously published results (Grimi et al., 2014). The poor yield of proteins for PEE and  
201 US assisted extractions can be explained by the following arguments. The microalgal proteins are located  
202 within different parts of the cells, including the cell wall, cytoplasm, chloroplast and all organelles inside  
203 the barrier of the cell wall (Safi et al., 2015). The “gentle” treatments, like PEE or US, can partially assist  
204 a release of the proteins present in cytoplasm or inside weak organelles. The complete extraction proteins  
205 require more serious disintegration of cells with applications of stronger techniques like bead milling or  
206 high pressure homogenization (Pataro et al., 2017). However, application of severe disruption techniques  
207 may induce significant to biological macromolecules (Günerken et al., 2015).

208         Among the tested species, *P. tricornutum* demonstrated the highest extraction yields of  
209 carbohydrates and proteins, while the lowest extraction yields were observed for *P. kessleri*. This possibly  
210 reflects the differences in resistance of cell walls against physical damages for these species. For PEF and  
211 US treatments, the values of  $Y_c$  and  $Y_p$  were arranged the row *P. tricornutum* > *Nannochloropsis* sp. > *P.*  
212 *kessleri*, whereas for HVED treatment they were arranged the row *Nannochloropsis* sp. > *P.*  
213 *tricornutum* > *P. kessleri* for carbohydrates and in the row *P. tricornutum* > *P. kessleri* > *Nannochloropsis*  
214 sp. for proteins. This extraction sensibility for different physical treatment techniques can reflect that the

215 differences in cell structure (e.g. size, shape, cell-wall structure and location of bio-molecules) in the  
216 tested microalgal species.

### 217 3.2 Extraction kinetic of pigments

218 **Fig. 4** presents kinetics of chlorophyll *a*,  $C_{ch}$ , extraction in the EtOH (95%, v/v) for different  
219 microalgae with application of different physical pretreatments. The value of  $C_{ch}$  increased with the  
220 increase of extraction time,  $t$ . For all species, at relatively long time ( $t \approx 24$  h), the US treatment was most  
221 efficient. HVED resulted in the approximately extraction efficiency as compared with PEF. The similar  
222 tendencies were observed for all times of extraction for *P. tricornutum*, and *P. kessleri*, but for  
223 *Nannochloropsis* sp. at  $t < 700$  min, the extraction efficiencies for pulsed electric energy treatments were  
224 more effective than for US treatment (**Fig. 4**).

225 The analysis of the experimental data showed that the extraction behavior of chlorophyll *a* after PEF,  
226 HVED, and US treatments followed the different kinetics. The extraction assisted by pulsed electric  
227 energy (PEF and HVED) can be fitted using the first-order exponential equation:

$$C_{ch} = C_{ch}^m [1 - \exp(-t/\tau)] \quad (5)$$

228 where  $C_{ch}$  is the content of chlorophyll *a* in the course of extraction;  $C_{ch}^m$  is the maximum value of  $C_{ch}$  for  
229 long extraction times;  $t$  is the time of extraction, and  $\tau$  is the effective extraction time.

230 The extraction of chlorophyll *a* assisted by US occurred in two stages and can be fitted by the  
231 following two-exponential law:

$$C_{ch} = C_{ch}^m [C_f^* (1 - \exp(-t/\tau_f)) + (1 - C_f^*) (1 - \exp(-t/\tau))] \quad (6)$$

232 where  $\tau_f$  and  $\tau$  correspond to the effective extraction times for the first (fast) and second (slow) stages,  
233 respectively. Here,  $C_f^* = C_f/C_{ch}^m$ ,  $C_f$  is the maximum concentration extracted during the first (fast) stage.

234 The dashed lines in the Fig. 4 correspond to the fittings of the experimental data (symbols) using  
235 one- exponential (Eq. (5) for PEE) and two-exponential (Eq. (6) for US) laws. In all cases the  
236 determination coefficients of fitting were rather high (in the interval  $R^2 = 0.96-0.99$ ).

237 The corresponding parameters evaluated for one-exponential (PEE) and two-exponential (US) laws  
238 for different species are presented in Supplementary materials (Table S1). For US assisted extraction the  
239 duration of the second (slow) stage was significantly higher of the first one ( $\tau_2 > \tau_1$ ). For *Nannochloropsis*  
240 sp. and *P. kessleri* the relative fraction of extracted chlorophyll *a* during the fast stage was rather small  
241 ( $C_f^* = 0.02-0.05$ ) and it was  $C_f^* = 0.52 \pm 0.04$  for *P. tricornutum*.

242 The obtained data clearly demonstrated the quite different behaviors of extraction kinetic of  
243 chlorophyll *a* with assistance of pulsed electric energy (PEF and HVED) or US treatments. The similar  
244 two-exponential behavior was previously observed for aqueous extraction assisted by US fennel tissue  
245 (Moubarik, El-Belghiti, & Vorobiev, 2011). The two stages in US assisted extraction can reflect the  
246 presence of pigments with different binding inside the cells and to the cell walls of the microalgal species.  
247 The first (fast) stage can be attributed to the release of some portion of weakly coupled pigments  
248 (possibly coupled with cell walls), while the second (slow) stage can be related with extraction of  
249 remained pigments from interior cell organelles. The values of relative concentration extracted during the  
250 first (fast) stage,  $C_f^*$ , can be arranged in the rows of *P. tricornutum* > *Nannochloropsis* sp. > *P.*  
251 *kessleri*. This order was in line with the order of extraction efficiencies of carbohydrates and proteins by  
252 US treatment. It possibly reflects the more fragile cell wall structure of *P. tricornutum* as compared to that  
253 for *Nannochloropsis* sp. or *P. kessleri*. The cell walls of *P. tricornutum* are composed of sulfated  
254 glucomannan (Francius et al., 2008) and they have more fragile structure as compare with cell walls of

255 *Nannochloropsis* sp. and *P. kessleri* mainly composed of cellulose and hemicelluloses (Payne &  
256 Rippingale, 2000).

257 To compare the efficiencies of extraction for untreated (U), physically treated (by PEF, HVED, and  
258 US) samples, the ratios  $F = C_{ch}^m / C_{ch}^{max}$  ( $C_{ch}^{max}$  is the total chlorophyll *a* content in microalgae) have been  
259 evaluated. **Fig. 5** presents the values of *F* for studied microalgal species.

260 For all studied microalgal species, the values of *F* can be arranged in the same row as for extraction  
261 yields of proteins (U < PEF < HVED < US). The highest extraction efficiency (18-40%) was observed for  
262 US assisted extraction. The similar effect of US treatment on extraction of proteins and pigments can  
263 reflect formation of protein-pigment complexes. The increased aqueous extraction of proteins  
264 supplemented with extraction of pigments was previously observed for application of US treatment of  
265 microalgal suspension (Parniakov et al., 2015).

266 Note that the highest content of chlorophyll *a* was observed in the raw *P. kessleri*, and it was  
267 smallest in the raw *P. tricornutum* (**Fig. 5**). However, for each tested physical method, the better  
268 extraction efficiency was observed from *Nannochloropsis* sp. as compared with *P. tricornutum* and *P.*  
269 *kessleri*. For example, the highest value of *F* ( $\approx 40\%$ ) obtained from *Nannochloropsis* sp. after US  
270 pretreatment was almost 2-fold and 2.6-fold higher than those obtained from *P. tricornutum* and *P.*  
271 *kessleri*.

272 **Fig. 6** compares the extraction time of slow stage,  $\tau$ , for different microalgal species for untreated  
273 (U), and PEF, HVED, and US (slow stage) treated samples. For green microalgae (*Nannochloropsis* sp.  
274 and *P. kessleri*), the value of  $\tau$  can be arranged in the row of U < PEF < US < HVED. The similar order  
275 was observed for both the values of *F* (**Fig. 5**) and  $\tau$  (**Fig. 6**). However, for *P. tricornutum*, the more

276 complicated behaviour was observed. Here, the extraction efficiency was highest for US treatment  
277 whereas the longest extraction time was observed for PEF treatment. The observed behavior reflect the  
278 sensibility of extraction efficiency upon the physical treatment and type of microalgal species.

#### 279 **4. Conclusions**

280 This study compares the extraction efficiencies of water-soluble (carbohydrates and proteins) and -  
281 insoluble (chlorophyll *a*) bio-molecules assisted by PEF, HVED and US techniques. The extraction  
282 efficiency arranged in the rows of U < PEF < US < HVED (for carbohydrates) and U < PEF < HVED <  
283 US (for proteins) was observed for all tested microalgal species. PEF treatment demonstrated the smallest  
284 efficiency for extraction of bio-molecules. The kinetics of extraction of chlorophyll *a* in EtOH solution  
285 was described using one-exponential (PEF and HVED) and two-exponential (US) equations. Significant  
286 differences in extraction behavior were observed for green microalgae (*Nannochloropsis* sp. and *P.*  
287 *kessleri*) and diatom (*P. tricornutum*). For *Nannochloropsis* sp. and *P. kessleri*, the US treatment was the  
288 most effective for extraction of chlorophyll *a*, but the longest extraction times were required with  
289 application of this technique. The observed differences for studied species can reflect the more fragile cell  
290 wall structure for *P. tricornutum* as compared with *Nannochloropsis* sp. or *P. kessleri*.

#### 291 **Declaration of competing Interest**

292 None.

#### 293 **Acknowledgments**

294 Rui Zhang would like to acknowledge the financial support of China Scholarship Council for thesis  
295 fellowship. The authors would like to thank Mrs. Laurence Lavenant for their technical assistance.

296

297 **References**

- 298 Alhattab, M., Kermanshahi-Pour, A., & Brooks, M. S.-L. (2019). Microalgae disruption techniques for  
299 product recovery: influence of cell wall composition. *Journal of Applied Phycology*, 31(1), 61–88.
- 300 Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of  
301 protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1-2), 248–254.
- 302 D’Alessandro, E. B., & Antoniosi Filho, N. R. (2016). Concepts and studies on lipid and pigments of  
303 microalgae: a review. *Renewable and Sustainable Energy Reviews*, 58, 832–841.
- 304 Da Silva Ferreira, V., & Sant’Anna, C. (2017). Impact of culture conditions on the chlorophyll content of  
305 microalgae for biotechnological applications. *World Journal of Microbiology and Biotechnology*,  
306 33(1), 20.
- 307 Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. t, & Smith, F. (1956). Colorimetric method for  
308 determination of sugars and related substances. *Analytical Chemistry*, 28(3), 350–356.
- 309 Duongbia, N., Chaiwongsar, S., Chaichana, C., & Chaiklangmuang, S. (2019). Acidic hydrolysis  
310 performance and hydrolyzed lipid characterizations of wet *Spirulina platensis*. *Biomass Conversion  
311 and Biorefinery*, 9(2), 305–319.
- 312 Francius, G., Tesson, B., Dague, E., Martin-Jézéquel, V., & Dufrêne, Y. F. (2008). Nanostructure and  
313 nanomechanics of live *Phaeodactylum tricornutum* morphotypes. *Environmental Microbiology*,  
314 10(5), 1344–1356.
- 315 Garcia, E. S., Lo, C., Eppink, M. H. M., Wijffels, R. H., & van den Berg, C. (2019). Understanding mild  
316 cell disintegration of microalgae in bead mills for the release of biomolecules. *Chemical  
317 Engineering Science*, 203, 380–390.
- 318 Gerde, J. A., Montalbo-Lomboy, M., Yao, L., Grewell, D., & Wang, T. (2012). Evaluation of microalgae  
319 cell disruption by ultrasonic treatment. *Bioresource Technology*, 125, 175–181.
- 320 Gonzalez-Balderas, R. M., Velasquez-Orta, S. B., Valdez-Vazquez, I., & Ledesma, M. T. O. (2020).  
321 Intensified recovery of lipids, proteins, and carbohydrates from wastewater-grown microalgae  
322 *Desmodesmus* sp. by using ultrasound or ozone. *Ultrasonics Sonochemistry*, 62, 104852.
- 323 Grimi, N., Dubois, A., Marchal, L., Jubeau, S., Lebovka, N. I., & Vorobiev, E. (2014). Selective  
324 extraction from microalgae *Nannochloropsis* sp. using different methods of cell disruption.  
325 *Bioresource Technology*, 153, 254–259.

- 326 Günerken, E., D'Hondt, E., Eppink, M. H. M., Garcia-Gonzalez, L., Elst, K., & Wijffels, R. H. (2015).  
327 Cell disruption for microalgae biorefineries. *Biotechnology Advances*, 33(2), 243–260.
- 328 Guihéneuf, F., Leu, S., Zarka, A., Khozin-Goldberg, I., Khalilov, I., & Boussiba, S. (2011). Cloning and  
329 molecular characterization of a novel acyl-CoA: diacylglycerol acyltransferase 1-like gene  
330 (PtDGAT1) from the diatom *Phaeodactylum tricornutum*. *The FEBS Journal*, 278(19), 3651–3666.
- 331 Henriques, M., Silva, A., & Rocha, J. (2007). Extraction and quantification of pigments from a marine  
332 microalga: a simple and reproducible method. *Communicating Current Research and Educational*  
333 *Topics and Trends in Applied Microbiology Formatex*, 2, 586–593.
- 334 Hosikian, A., Lim, S., Halim, R., & Danquah, M. K. (2010). Chlorophyll extraction from microalgae: a  
335 review on the process engineering aspects. *International Journal of Chemical Engineering*, 2010,  
336 391632 (11 pages).
- 337 Khili, M. (2013). *Characterization of Value Added Proteins and Lipids form Microalgae*. Virginia Tech.
- 338 Lorenzo-Hernando, A., Ruiz-Vegas, J., Vega-Alegre, M., & Bolado-Rodriguez, S. (2019). Recovery of  
339 proteins from biomass grown in pig manure microalgae-based treatment plants by alkaline  
340 hydrolysis and acidic precipitation. *Bioresource Technology*, 273, 599–607.
- 341 Macías-Sánchez, M. D., Mantell Serrano, C., Rodríguez Rodríguez, M., de la Ossa, E., Lubián, L. M., &  
342 Montero, O. (2008). Extraction of carotenoids and chlorophyll from microalgae with supercritical  
343 carbon dioxide and ethanol as cosolvent. *Journal of Separation Science*, 31(8), 1352–1362.
- 344 Moubarik, A., El-Belghiti, K., & Vorobiev, E. (2011). Kinetic model of solute aqueous extraction from  
345 Fennel (*Foeniculum vulgare*) treated by pulsed electric field, electrical discharges and ultrasonic  
346 irradiations. *Food and Bioproducts Processing*, 89(4), 356–361.
- 347 Parniakov, O., Barba, F. J., Grimi, N., Marchal, L., Jubeau, S., Lebovka, N., & Vorobiev, E. (2015a).  
348 Pulsed electric field and pH assisted selective extraction of intracellular components from  
349 microalgae *Nannochloropsis*. *Algal Research*, 8, 128–134.
- 350 Parniakov, O., Barba, F. J., Grimi, N., Marchal, L., Jubeau, S., Lebovka, N., & Vorobiev, E. (2015b).  
351 Pulsed electric field assisted extraction of nutritionally valuable compounds from microalgae  
352 *Nannochloropsis* spp. using the binary mixture of organic solvents and water. *Innovative Food*  
353 *Science & Emerging Technologies*, 27, 79–85.
- 354 Pataro, G., Goettel, M., Straessner, R., Gusbeth, C., Ferrari, G., & Frey, W. (2017). Effect of PEF  
355 treatment on extraction of valuable compounds from microalgae *C. vulgaris*. *Chemical Engineering*  
356 *Transactions*, 57, 67–72.
- 357 Payne, M. F., & Rippingale, R. J. (2000). Evaluation of diets for culture of the calanoid copepod  
358 *Gladioferens imparipes*. *Aquaculture*, 187(1-2), 85–96.

- 359 Phelippe, M., Goncalves, O., Thouand, G., Cogne, G., & Laroche, C. (2019). Characterization of the  
360 polysaccharides chemical diversity of the cyanobacteria *Arthrospira platensis*. *Algal Research*, 38,  
361 101426.
- 362 Postma, P. R., Suarez-Garcia, E., Safi, C., Yonathan, K., Olivieri, G., Barbosa, M. J., ... Eppink, M. H. M.  
363 (2017). Energy efficient bead milling of microalgae: Effect of bead size on disintegration and  
364 release of proteins and carbohydrates. *Bioresource Technology*, 224, 670–679.
- 365 Reboloso-Fuentes, M. M., Navarro-Pérez, A., Garc'ia-Camacho, F., Ramos-Miras, J. J., & Guil-  
366 Guerrero, J. L. (2001). Biomass nutrient profiles of the microalga *Nannochloropsis*. *Journal of*  
367 *Agricultural and Food Chemistry*, 49(6), 2966–2972.
- 368 Ritchie, R. J. (2006). Consistent sets of spectrophotometric chlorophyll equations for acetone, methanol  
369 and ethanol solvents. *Photosynthesis Research*, 89(1), 27–41.
- 370 Rivera, E. C., Montalescot, V., Viau, M., Drouin, D., Bourseau, P., Frappart, M., ... Couallier, E. (2018).  
371 Mechanical cell disruption of *Parachlorella kessleri* microalgae: Impact on lipid fraction  
372 composition. *Bioresource Technology*, 256, 77–85.
- 373 Safi, C., Frances, C., Ursu, A. V., Laroche, C., Pouzet, C., Vaca-Garcia, C., & Pontalier, P.-Y. (2015).  
374 Understanding the effect of cell disruption methods on the diffusion of *Chlorella vulgaris* proteins  
375 and pigments in the aqueous phase. *Algal Research*, 8, 61–68.
- 376 Sankaran, R., Manickam, S., Yap, Y. J., Ling, T. C., Chang, J.-S., & Show, P. L. (2018). Extraction of  
377 proteins from microalgae using integrated method of sugaring-out assisted liquid biphasic flotation  
378 (LBF) and ultrasound. *Ultrasonics Sonochemistry*, 48, 231–239.
- 379 Sedighi, M., Jalili, H., Darvish, M., Sadeghi, S., & Ranaei-Siadat, S.-O. (2019). Enzymatic hydrolysis of  
380 microalgae proteins using serine proteases: A study to characterize kinetic parameters. *Food*  
381 *Chemistry*, 284, 334–339.
- 382 Skorupskaite, V., Makareviciene, V., Sendzikiene, E., & Gumbyte, M. (2019). Microalgae *Chlorella* sp.  
383 cell disruption efficiency utilising ultrasonication and ultrahomogenisation methods. *Journal of*  
384 *Applied Phycology*, 1–6.
- 385 Yu, X., Gouyo, T., Grimi, N., Bals, O., & Vorobiev, E. (2016). Pulsed electric field pretreatment of  
386 rapeseed green biomass (stems) to enhance pressing and extractives recovery. *Bioresource*  
387 *Technology*, 199, 194–201.
- 388 Zhang, R., Grimi, N., Marchal, L., Lebovka, N., & Vorobiev, E. (2019). Effect of ultrasonication, high  
389 pressure homogenization and their combination on efficiency of extraction of bio-molecules from  
390 microalgae *Parachlorella kessleri*. *Algal Research*, 40, 101524.

391

392 **Figure captions**

393 **Fig. 1** Schema of the applied experimental procedures.

394 **Fig. 2.** Schematic representation of pulsed electric energy (PEF and HVED) treatment chambers (a), and  
395 pulsed protocols (b) used for treatment of microalgal suspensions.

396 **Fig. 3.** The extraction yields of carbohydrates,  $Y_c$ , and proteins,  $Y_p$ , in the supernatants, obtained from  
397 untreated (U) and physically (PEF, HVED, US) treated samples for different microalgae species. The total  
398 energy consumption of physical treatments was the same,  $W \approx 530$  kJ/kg suspension.

399 **Fig. 4.** The extraction kinetics of chlorophyll  $a$ ,  $C_{ch}$ , for different microalgal species for physically (PEF,  
400 HVED, US) treated samples. The dashed lines in the Fig. 4 correspond to the fittings of the experimental  
401 data (symbols) using one-exponential (Eq. (5) for PEE) and two-exponential (Eq. (6) for US) laws.

402 **Fig. 5.** Maximum extraction efficiency of chlorophyll  $a$ ,  $F$ , for different microalgal species for untreated  
403 (U) and physically (PEF, HVED, US) treated samples.

404 **Fig. 6.** Effective extraction time,  $\tau$ , for different microalgal species for untreated (U) and PEF, HVED, US  
405 (slow stage) treated samples.

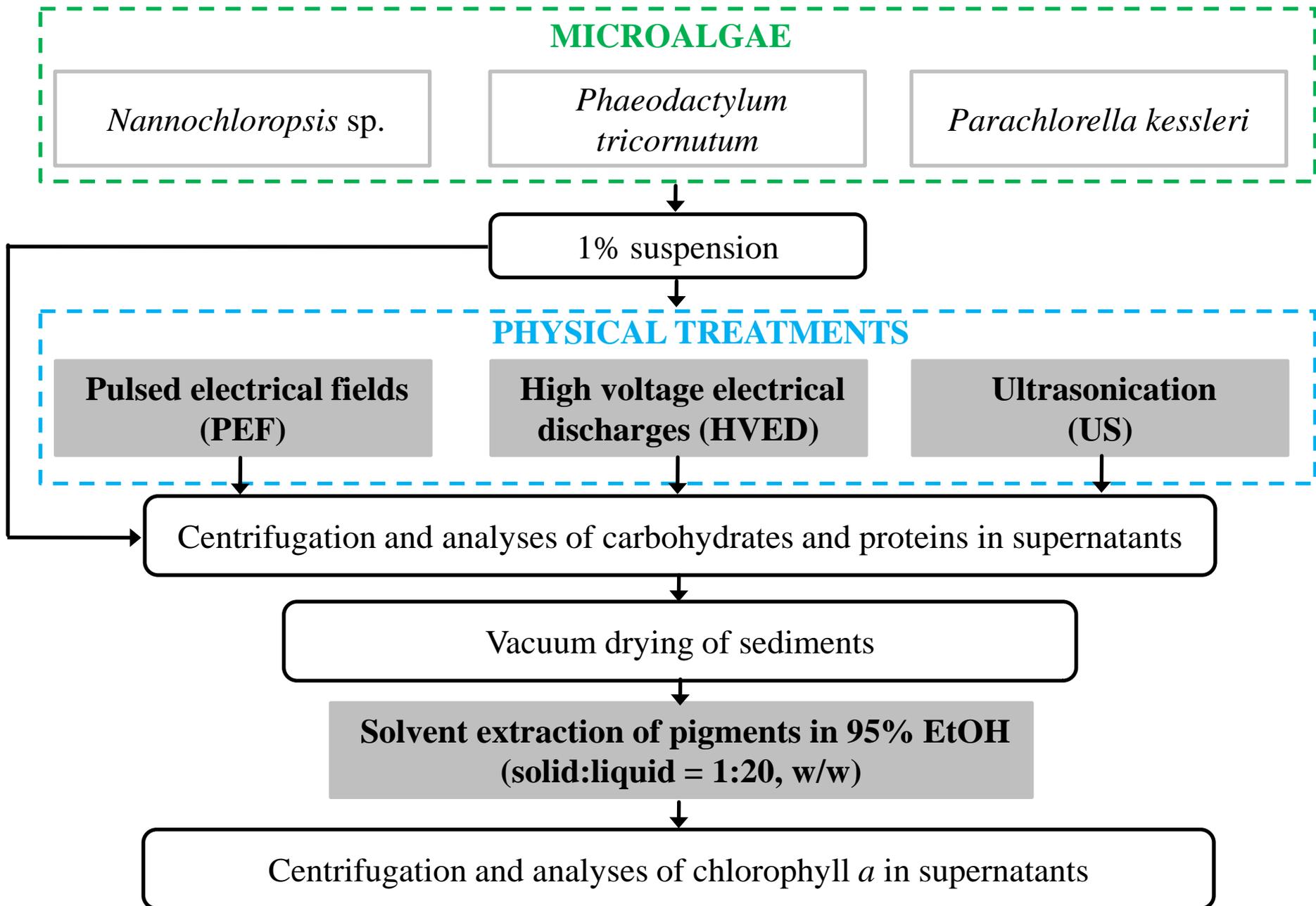
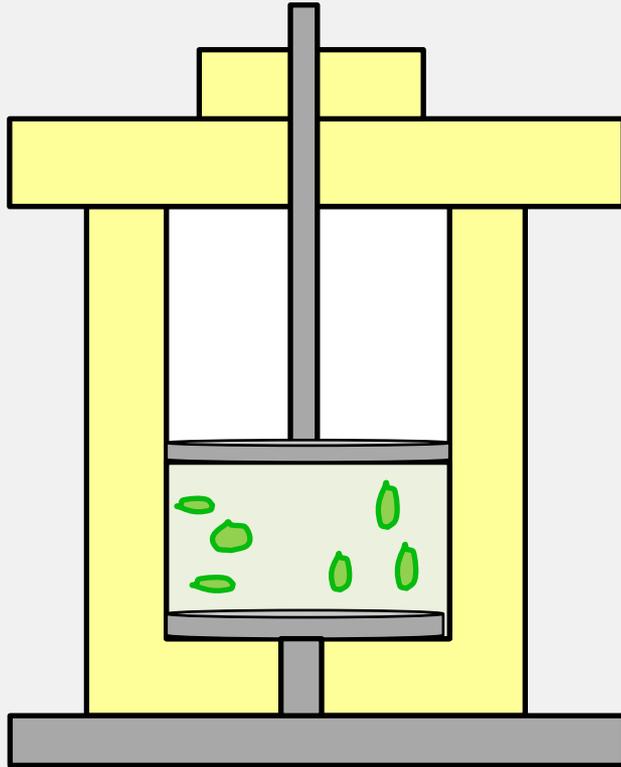


Fig. 1

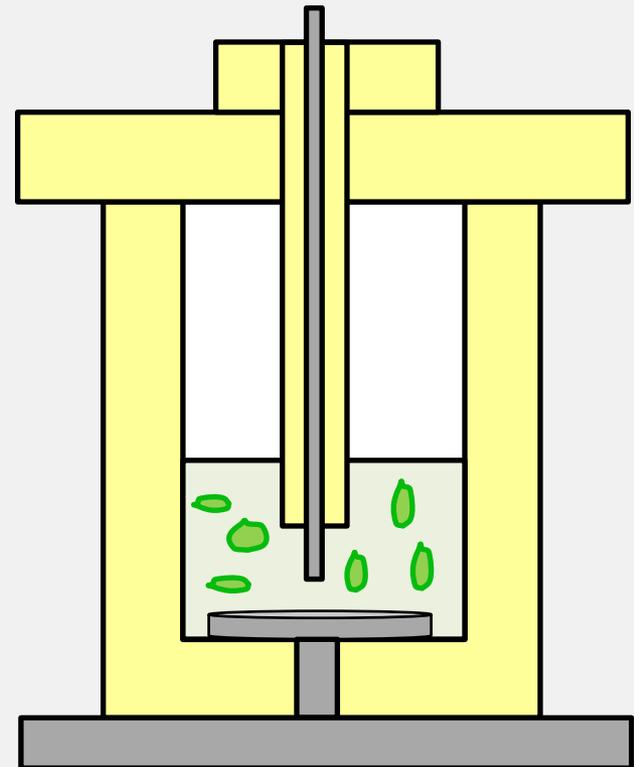
a)

*Plate to plate geometry*



**PEF**

*Needle to plate geometry*



**HVED**

b)

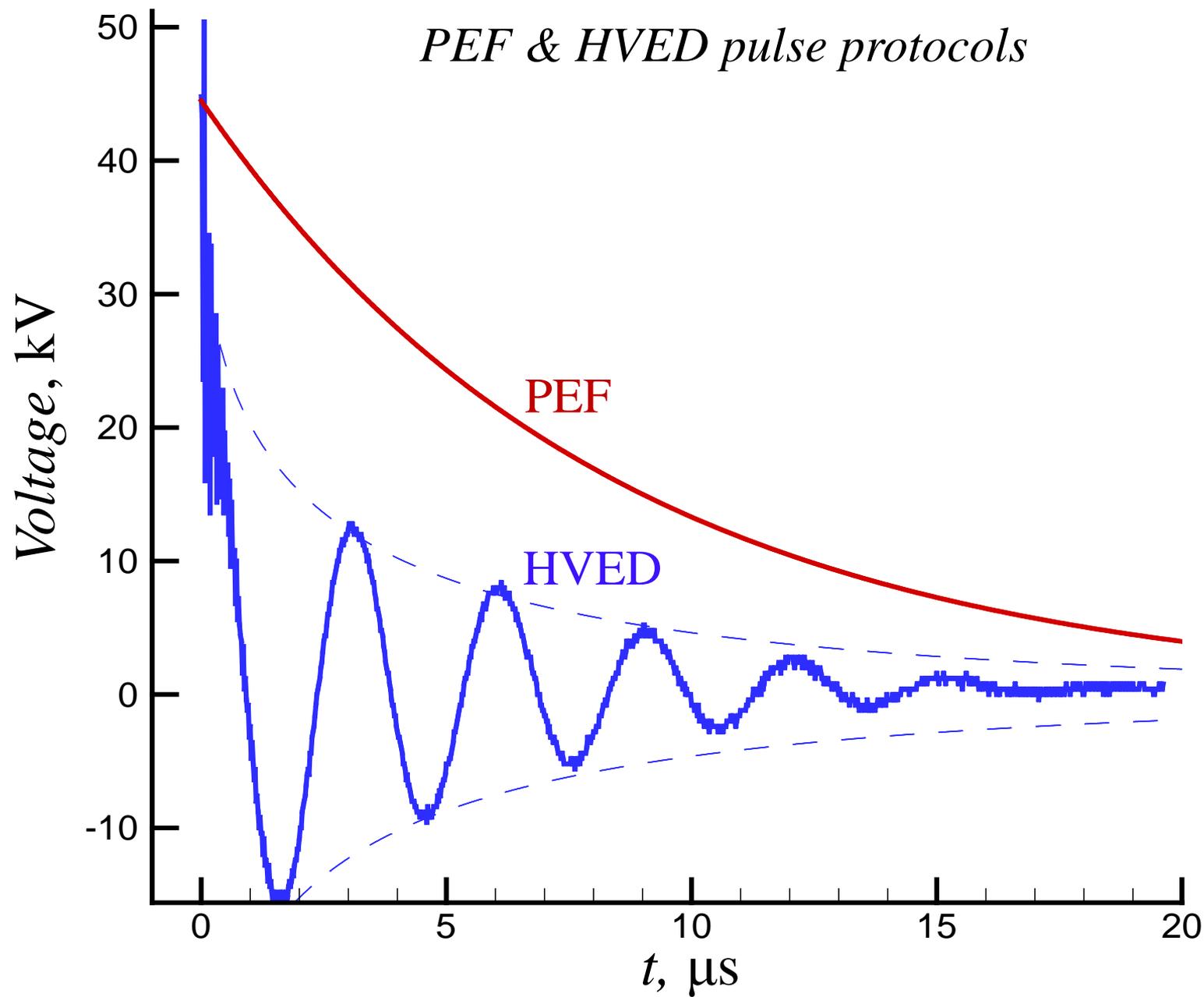


Fig. 2b

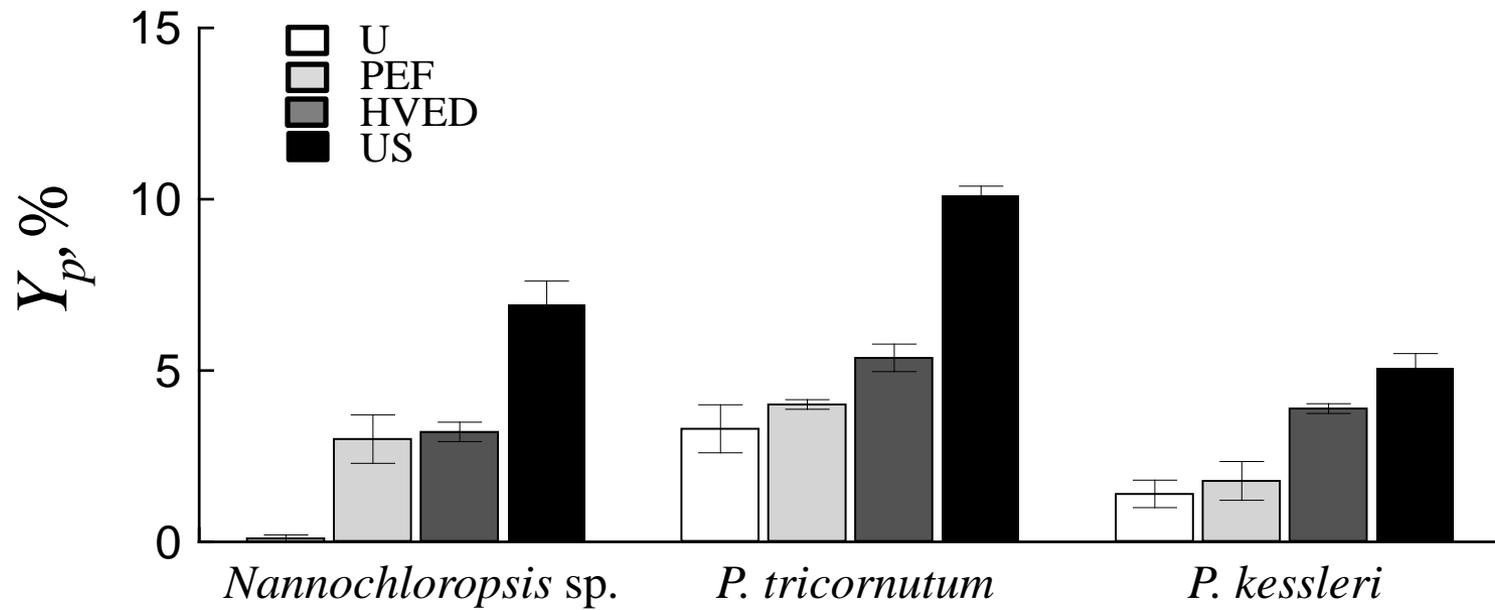
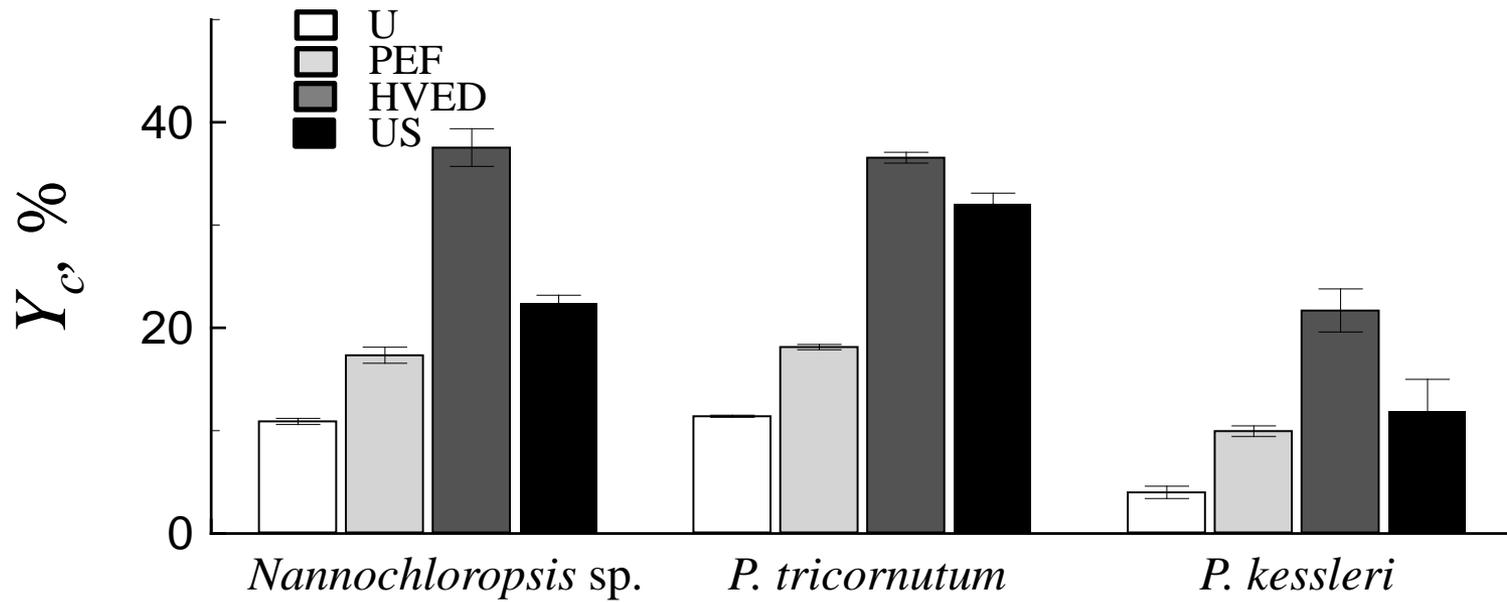


Fig. 3

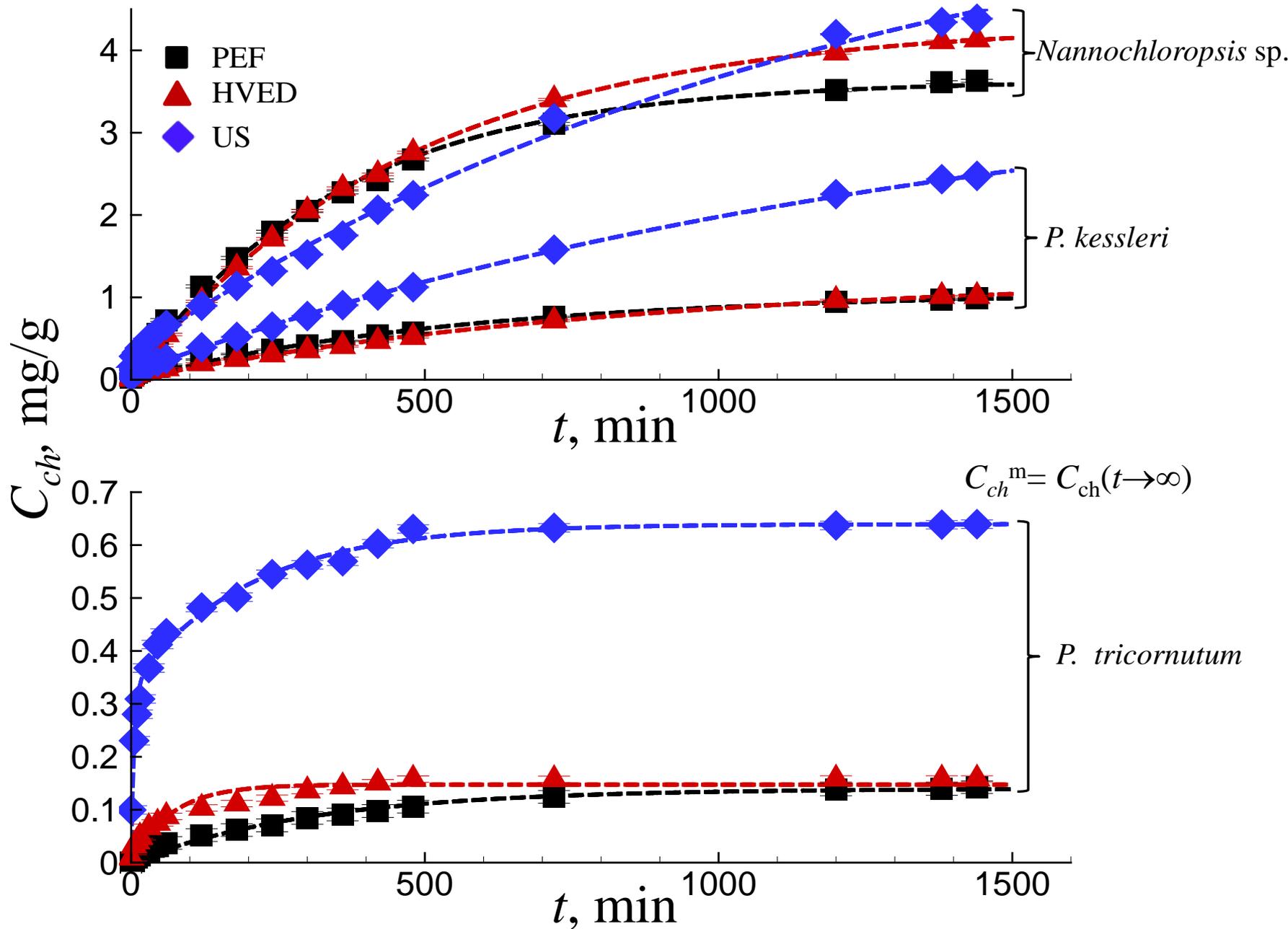


Fig. 4

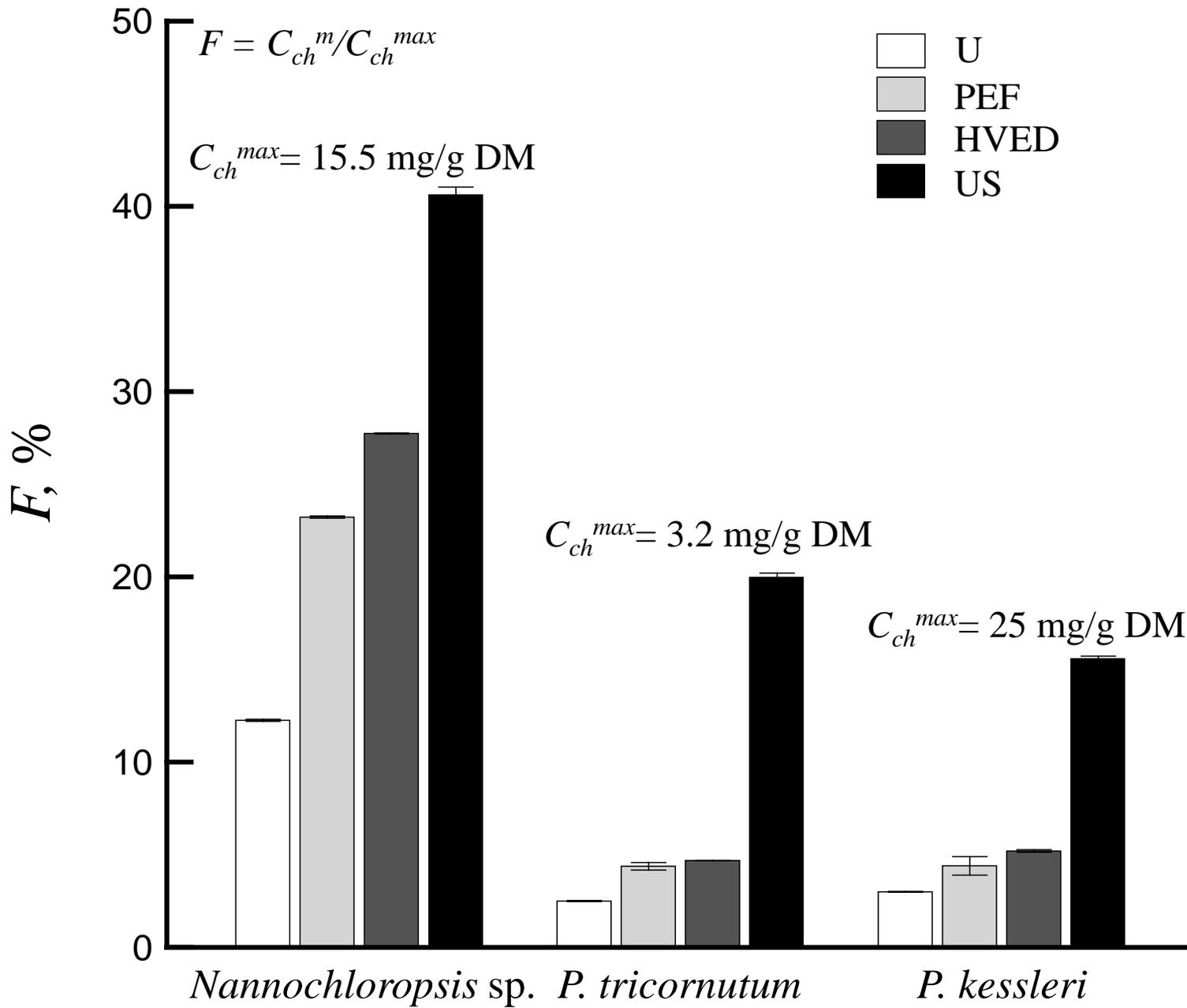


Fig. 5

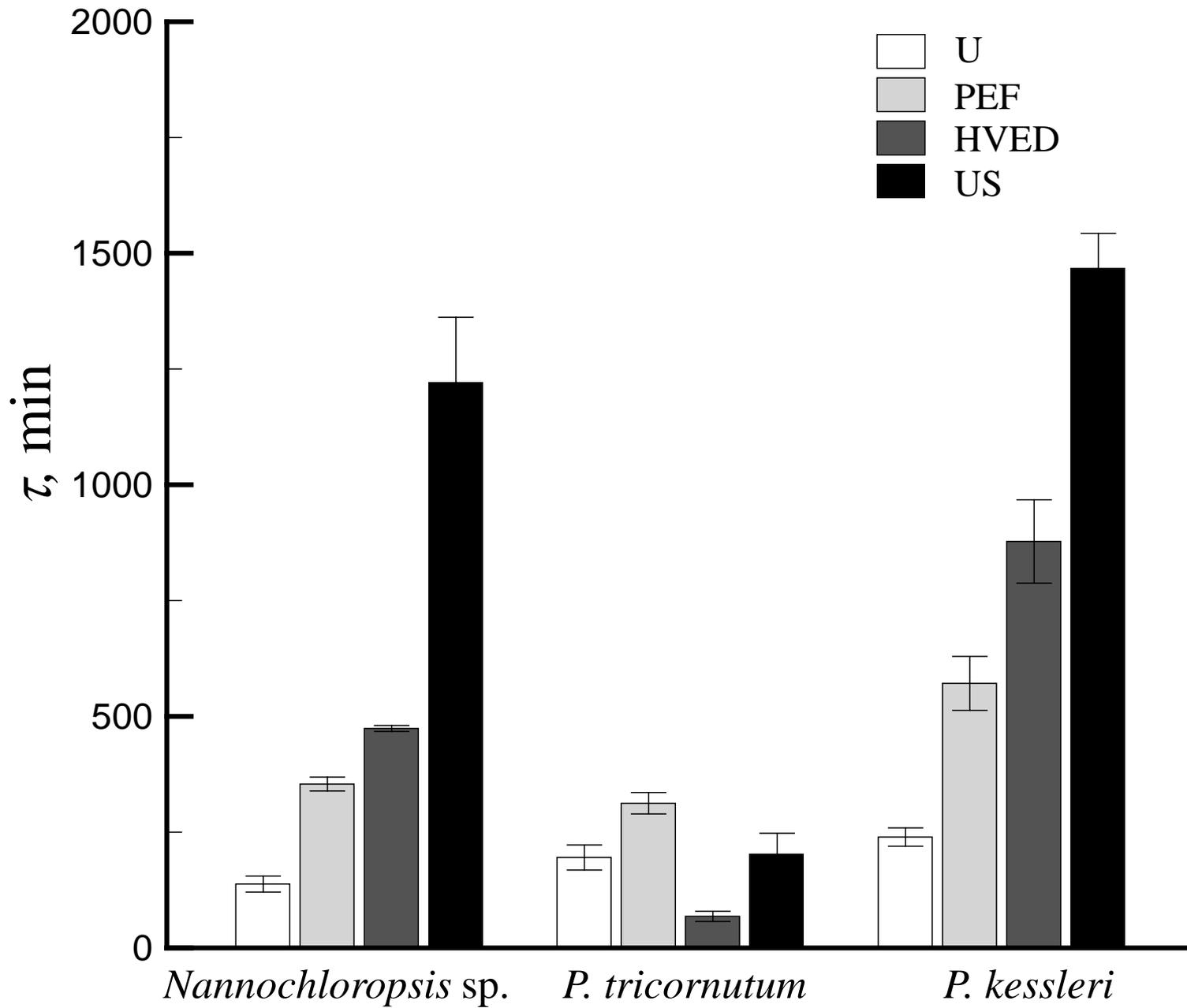


Fig. 6