



HAL
open science

Two-step procedure for selective recovery of bio-molecules from microalga *Nannochloropsis oculata* assisted by high voltage electrical discharges

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

► To cite this version:

Rui Zhang, Luc Marchal, Nikolai Lebovka, Eugène Vorobiev, Nabil Grimi. Two-step procedure for selective recovery of bio-molecules from microalga *Nannochloropsis oculata* assisted by high voltage electrical discharges. *Bioresource Technology*, 2020, 302, pp.122893. 10.1016/j.biortech.2020.122893 . hal-02973713

HAL Id: hal-02973713

<https://hal.science/hal-02973713>

Submitted on 21 Jul 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 **Two-step procedure for selective recovery of bio-molecules**
2 **from microalga *Nannochloropsis oculata* assisted by high voltage**
3 **electrical discharges**

4 Rui Zhang^{1*}, Luc Marchal², Nikolai Lebovka^{1,3}, Eugène Vorobiev¹, Nabil Grimi

5
6 ¹Sorbonne University, Université de Technologie de Compiègne, ESCOM, EA 4297

7 TIMR, Centre de recherche Royallieu - CS 60319 - 60203 Compiègne cedex, France

8 ²LUNAM Université, CNRS, GEPEA, Université de Nantes, UMR6144, CRTT,

9 Boulevard de l'Université, BP 406, 44602 Saint-Nazaire Cedex, France ;

10 ³Institute of Biocolloidal Chemistry named after F. D. Ovcharenko, NAS of Ukraine, 42,

11 blvr. Vernadskogo, Kyiv 03142, Ukraine

12
13 *Corresponding Author Address:

14 Mrs. Rui Zhang

15 ¹Sorbonne University, Université de Technologie de Compiègne, ESCOM, EA 4297

16 TIMR, Centre de recherche Royallieu - CS 60319 - 60203 Compiègne cedex, France

17 E-mail address: rui.zhang@utc.fr

18

19 **Abstract**

20 Two-step procedure with the initial aqueous extraction from raw microalgae
21 *Nannochloropsis oculata* and secondary organic solvent extraction from vacuum dried
22 (VD) microalgae were applied for selective recovery of bio-molecules. The effects of
23 preliminary aqueous washing and high voltage electrical discharges (HVED, 40 kV/cm,
24 4 ms pulses) were tested. The positive effects of HVED treatment and washing on
25 selectivity of aqueous extraction of ionics and other water-soluble compounds
26 (carbohydrates, proteins and pigments) were observed. Moreover, the HVED treatment
27 allowed improving the kinetic of vacuum drying, and significant effects of HVED
28 treatment on organic solvent extraction of chlorophylls, carotenoids and lipids were
29 determined. The proposed two-step procedure combining the preliminary washing,
30 HVED treatment and aqueous/organic solvents extraction steps are useful for selective
31 extraction of different bio-molecules from microalgae biomass.

32 **Keywords:** Microalgae; High voltage electrical discharges; Vacuum drying; Pigments;
33 Lipids

34

35 1. Introduction

36 Nowadays, the recovery of bio-molecules from microalgae has attracted wide
37 attention of academic and industrial researchers (Mittal and Raghavarao, 2018).
38 Microalgae have high growth rate, photosynthetic efficiency, worldwide distribution,
39 and valuable bio-contents (Daneshvar et al., 2018). Due to high proportion of proteins
40 and micronutrients in extracts (Buchmann et al., 2019), they present promising source
41 for food and feed production. Moreover, the extracted pigments and polyphenols from
42 microalgae can be used in cosmetics and pharmaceutical industries (Rivera et al., 2018),
43 and their lipid extracts can serve as raw material for biofuels (Talebi et al., 2013).
44 *Nannochloropsis* sp. is a marine green microalgae belonging to the Eustigmataceae
45 family (Parniakov et al., 2015). The major photosynthetic pigments are violaxanthin,
46 vaucheraxanthin, and chlorophylls (Reboloso-Fuentes et al., 2001). In favorable
47 growing conditions (with adjustable temperature, salinity and additives), they can also
48 accumulate considerable amounts of lipids ranging from 12 to 60% w/w (Chiu et al.,
49 2009; Doan and Obbard, 2015; Mitra et al., 2015). However, the extraction of bio-
50 molecules from *Nannochloropsis* sp. is not easy task. Their cells are near spherical with
51 relatively small size ($\approx 2.5 \mu\text{m}$) and they are covered by rather thick rigid walls (≈ 60 -
52 110 nm) (Gerken et al., 2013). To facilitate extraction of intracellular compounds,
53 different chemical, enzymatic (Zhu et al., 2018a) and physical methods (Zhu et al.,
54 2018b) have been tested (for a recent review see (Zhang et al., 2018b)).

55 The applications of pulsed electric energy (pulsed electric fields (PEF) (Parniakov

56 et al., 2015a, 2015b) and high voltage electrical discharges (HVED)) (Grimi et al., 2014)
57 for recovery intracellular compounds from *Nannochloropsis* sp. have been reported. The
58 PEF provoke electroporation of cell membranes, while the HVED can provoke the
59 damage of cell walls due to electrical breakdown and different secondary phenomena
60 (liquid turbulence, intense mixing, shock waves, and bubble cavitation, etc) (Barba et al.,
61 2015). They can be easily done for algal slurries with the high moisture content of (\approx
62 80% wt). However, the effective extraction of hydrophobic bio-molecules, such as
63 pigments, lipids and phenolic compounds requires applications of more complex
64 techniques, including organic solvents extraction (Barba et al., 2015), high pressure
65 homogenization (Zhang et al., 2018a) and ultrasonication (Zhang et al., 2019), etc.

66 Moreover, the extraction of hydrophobic bio-molecules requires drying of algal
67 slurries with significant reducing of a moisture content up to \approx 10% that is time and
68 energy consuming process (Ansari et al., 2018; Bagchi et al., 2015; Hosseinizand et al.,
69 2018). Vacuum drying (VD) has been recently applied for processing of algal cells
70 (Makkar et al., 2016). Effects of VD on seaweed *Pyropia orbicularis* at the pressure of
71 15 kPa and drying temperatures at 40-80 °C were investigated (Uribe et al., 2018). The
72 high recovery yields of total phenolic, carotenoids, phycoerythrin and phycocyanin were
73 demonstrated. Furthermore, the pre-treatment by pulsed electric energy can also affect
74 the efficiency of VD (Liu et al., 2018).

75 The main aim of this work was efficiency testing of the two-step procedure for
76 selective recovery of bio-molecules from microalga *Nannochloropsis oculata* (N.

77 *oculata*) assisted by HVED. The procedure combined washing with HVED treatment at
78 the initial aqueous extraction step, and VD before at the final non-aqueous extraction
79 step. The effects of HVED pre-treatment on the extraction efficiencies of hydrophilic
80 components (ionics, carbohydrates, proteins and water-soluble pigments) (first step),
81 and hydrophobic components (pigments and lipids) (second step) were investigated.
82 Moreover, the impact of HVED treatment on VD kinetics was evaluated for the first
83 time.

84 **2. Materials and methods**

85 *2.1 Microalgae*

86 Microalga *Nannochloropsis oculata* (*N. oculata*) (provided by AlgoSolis, Saint-
87 Nazaire, France) was obtained as a frozen paste. The moisture content of biomass was
88 measured at 105 °C for 24 h. Accounting for this content, the biomass, at the initial step,
89 was diluted with deionized water to obtain 5% dry matter (DM) concentration.

90 *2.2 Design of experiments*

91 **Fig. 1a** presents the schema of experiments applied in the present study. It includes
92 the preparation of samples (without or with preliminary washing), HVED treatment and
93 aqueous extraction (first step for extraction of water-soluble components), and VD of
94 sediments and organic solvent extraction (second step for extraction of pigments and
95 lipids).

96 2.2.1. Preparation of the samples

97 In the supplied biomass paste, the water-soluble components were present in the
98 extracellular aqueous solution. In order to evaluate effects of these components on
99 extraction efficiency for different applied procedures, the samples with and without
100 washing were prepared.

101 The initial samples with preliminary washing for 60 min were designed as S₀. The
102 aqueous extraction for 30 min was performed for biomass (both unwashed and washed)
103 (Fig. 1a). Samples processed without preliminary washing were designed as S₁
104 (untreated) and S₂ (HVED treated). Samples processed with preliminary washing were
105 designed as S₃ (untreated) and S₄ (HVED treated). In the first washing step, the
106 suspension was diluted to 1% DM, agitated at 150 rpm for 10 min, and centrifuged for
107 10 min at 4600 g. Then supernatant was removed and sediment was diluted to 1% DM
108 and the next washing step was applied. The duration of one washing step was 20 min.
109 The analysis of supernatant for presence ionic components, proteins, and carbohydrates
110 was done (see Section 2.3 for the details).

111 2.2.2. High voltage electrical discharges (HVED) treatment

112 HVED treatment was applied using a high voltage pulsed power 40 kV-10 kA
113 generator (Basis, Saint-Quentin, France). HVED treatment was done in a 1-L cylindrical
114 batch treatment cell with an electrode of needle-plate geometry (Fig. 1b). The distance
115 between stainless steel needle and grounded plate was fixed to 1 cm, which
116 corresponding to $E = 40$ kV/cm of electric field strength. HVED treatment comprised of

117 the application of n successive pulses ($n = 1-400$) and a pulse repetition rate of 0.5 Hz.
118 The total time of electrical treatment was 0.01-4 ms. This discharge protocol with $E =$
119 40 kV/cm and $n = 400$ was shown to be effective for extraction of water-soluble
120 proteins (Grimi et al., 2014). The damped oscillations with effective decay time $t_p \approx 10$
121 $\pm 0.1 \mu\text{s}$ were observed in HVED mode (Fig. 1b). The 2 min of pause was done after
122 each 100 pulses to maintain temperature elevation after HVED treatment never
123 exceeded 30 °C. The total operation time for HVED experiment is 30 min. The 200 g of
124 suspension of microalgae (5% DM) was used in this study (samples S₂ and S₄). The
125 samples without HVED treatment keep for 30 min was used as control (samples S₁ and
126 S₃). Then these samples were centrifuged at 14,100 g for 10 min using a MiniSpin Plus
127 Rotor F-45-12-11 (Eppendorf, France). The supernatants were used for analyzing of
128 extracts and the sediments were dehydrated by VD.

129 2.2.3. Vacuum drying (VD) of sediments

130 The sediments were mixed with absolute EtOH (2:1, w/w). The 10 g of mixture
131 were spread uniformly with 7 mm of initial thickness in Petri dishes (6 cm inner
132 diameter and 2.5 cm inner depth) and kept for VD. The absolute EtOH was added to
133 centrifuged samples to facilitate detachment of cells from the centrifugation glass tubes.
134 Then the EtOH was evaporated quickly from the samples. VD experiment was carried
135 out in a vacuum chamber (Cole-Parmer, USA) connected with a vacuum pump
136 (Rietschle, Germany). The pressure of drying chamber was maintained at 30 kPa and
137 the drying temperature was fixed at 50 °C. The initial temperature of samples ($\approx 25^\circ\text{C}$)

138 was measured before VD experiment. During the drying, the temperature inside centre
139 of the sample, T , was recorded in the online mode using a thermocouple (K-type, NiCr–
140 Ni). The weight of the sample, m , was measured using a balance (GF-600, A & D,
141 Japan).

142 The moisture ratio, MR , of the sample during the drying was calculated as follows:

$$MR = (m - m_f) / (m_i - m_f) \quad (1)$$

143 where m is the running weight of the sample, and the subscripts i and f refer to the initial
144 and final (completely dried) values, respectively. In experiments, the final (completely
145 dried) value was determined by oven drying samples at 105 °C for 24 h.

146 After VD processing, the dried biomass was respectively used for analysis of
147 pigments and lipids extraction.

148 *2.3 Analysis of extracts*

149 2.3.1 Aqueous extracts

150 The following aqueous suspensions were centrifuged at 14,100 g for 10 min. The
151 supernatants were used for analyzing microalgae extracts. All characterization
152 measurements were done at ambient temperature.

153 The extent of the releasing of ionic components was characterized by measurements
154 of the electrical conductivity by the instrument InoLab pH/cond Level 1 (WTW,
155 Weilheim, Germany). The soluble matter content (°Brix) was measured by a digital
156 Atago refractometer (PR-101, Atago, 50 Tokyo, Japan). For determination of dry weight
157 content (DW), 150 mL of supernatant was placed in glass beaker and dried in an oven at

158 105 °C for 24 h. The value of DW was gravimetrically determined by weighting the
159 samples before and after drying. The results were expressed as mg of dry matter/g of
160 supernatant. The contents of carbohydrates, C_c , and proteins, C_p , were determined using
161 a phenol-sulfuric acid (Dubois et al., 1956) and Bradford's method (Bradford, 1976),
162 respectively. The pigments were determined using spectroscopic-based techniques by
163 UV/vis spectrophotometer (Thermo Spectronic Genesys 20, Thermo Electron
164 Corporation, MA).

165 Briefly, for determination of the content of carbohydrates, D-glucose standard
166 provided by Sigma-Aldrich (Saint-Quentin Fallavier, France) was used for the
167 calibration curve. The 1 mL of supernatants (diluted if required), 0.1 mL of 5% phenol
168 solution and 5 mL of concentrated sulfuric acid were mixed in glass tubes. The mixture
169 was incubated at 20 °C for 20 min. Absorbance was measured using at the wavelength
170 of 490 nm.

171 For determination of the content of proteins, the diluted supernatant (0.1 mL) was
172 mixed 1 mL of Bradford Dye Reagent (Thermo Fisher, Kandel, Germany) and kept for
173 5 min. The absorbance was measured at the wavelength of 595 nm. Bovine serum
174 albumin (BSA) provided by Sigma-Aldrich (Saint-Quentin Fallavier, France) was used
175 for the calibration curve.

176 For determination of the content of pigments, the supernatant was mixed with
177 EtOH (95%, v/v) (50 μ L sample + 950 μ L 95% EtOH). The absorbances of chlorophyll
178 *a*, chlorophyll *b*, and total carotenoids were measured at the wavelengths of 664, 649

179 and 470 nm using 95% EtOH as blank. The concentrations of chlorophyll *a*, C_{ch}^a ,
180 chlorophyll *b*, C_{ch}^b , total chlorophylls, C_{ch} , and total carotenoids, C_{cr} , ($\mu\text{g pigment/mL}$
181 supernatant) were calculated using the following equations (Gerde et al., 2012):

$$C_{ch}^a = 13.36 \times A_1 - 5.19 \times A_2, \quad (2a)$$

$$C_{ch}^b = 27.43 \times A_2 - 8.12 \times A_1, \quad (2b)$$

$$C_{ch} = C_{ch}^a + C_{ch}^b, \quad (2c)$$

$$C_{cr} = (1000 \times A_3 - 2.13 \times C_{ch}^a - 97.64 \times C_{ch}^b) / 209, \quad (2d)$$

182 where A_1 , A_2 , A_3 are the absorbances measured at the wavelengths of 664, 649, and 470
183 nm, respectively.

184 The content of components released from microalgae was expressed as mg/g dry
185 microalgae.

186 2.3.2 Organic solvent extracts

187 For analysis of extraction of pigments, the biomass obtained by VD with a final MR
188 of 0.01 and 0.2 was diluted with 95% EtOH to solid-liquid ratio of 1: 20. The extraction
189 was studied for 8 h under the stirring at 150 rpm. To avoid any evaporation, the
190 extraction cells were covered with aluminum foil during the extraction process.

191 For analysis of extraction of lipids, the standard lipid extraction procedure was used
192 according to the “whole cell analysis” (WCA) method (Van Vooren et al., 2012). Briefly,
193 in order to prevent oxidation of lipids, the dried samples ($MR_f = 0.01$) were first mixed
194 with 20 μL distilled water and 10 μL butylated hydroxytoluene (BHT, 20 $\mu\text{g/uL}$) in
195 clean vials. Then the mixture was suspended in 6 mL of a chloroform/methanol (CHCl_3 :

196 MeOH, 2:1, v/v) mixture. Vials were maintained for 6 h in the dark under slow agitation.
197 After extraction, the organic and aqueous phases were separated and the solvent of the
198 extracts was evaporated under N₂ flux. 1 mL of CHCl₃/MeOH (2/1, v/v) mixture was
199 then added and stored at -20 °C until analysis. Total fatty acids (TFA) contents in the
200 lipid extracts were quantified by Gas Chromatography-Flame Ionization Detector (GC-
201 FID) (Agilent Technologies Inc., Santa Clara, CA) analysis. Fatty acid methyl ester
202 (FAME) contents in the lipid extracts were quantified after a transesterification step.
203 More details can be found in (Van Vooren et al., 2012). The values of the total lipids
204 content, C_l , and relative content of fatty acids (saturated fatty acids (SFA, all single
205 bonds between carbon atoms), monounsaturated fatty acids (MUFA, one double bond)
206 and polyunsaturated fatty acids (PUFA, at least two double bonds) were evaluated.

207 2.3.3 Content of bio-molecules in totally disintegrated cells

208 For total disintegration of cells, the biomass was grinded using a bead-beating
209 method at 30 Hz (MM400 mixer mill, Retsch GmbH & co. KG, Haan, Germany). In this
210 method, the cells are mechanically disrupted by ceramic beads in the reaction vials. For
211 determination of maximum content of proteins, carbohydrates, chlorophylls, and
212 carotenoids in microalgae the washing procedure was initially applied. The biomass was
213 diluted to 1% DM, agitated at 150 rpm for 10 min, and centrifuged for 10 min at 4600 g.
214 Then supernatant was removed and the washing procedure was repeated 3 times. Finally,
215 the sediment was separated and lyophilization was applied for 64 h at -20 °C using a
216 MUT 002A pilot freeze-drier (Cryotec, France). The final *MR* of the sample was 0.08.

217 Then the dried biomass was grinded in a wet mode. The dried biomass was initially
218 diluted with distilled water for analysis of proteins and carbohydrates and 95% EtOH
219 for analysis of chlorophylls and carotenoids. The solid-liquid ratio was 1: 20 and the
220 grinding was done for 15 min. To avoid overheating during the grindings the 15 s
221 pauses after each minute were applied. The maximum contents were obtained: $C_p =$
222 47.67 ± 0.83 mg/g DM (proteins), $C_c = 67.36 \pm 0.46$ mg/g DM (carbohydrates), $C_{ch} =$
223 1.17 ± 0.01 mg/g DM (total chlorophylls), $C_{cr} = 0.256 \pm 0.001$ mg/g DM (carotenoids).

224 2.4 Statistical analysis

225 Each experiment was replicated three to five times. The error bars, presented on the
226 figures, correspond to the standard deviations. One-way analysis of variance (ANOVA)
227 was used to determine significant differences ($p < 0.05$) among the samples with the
228 help of OriginPro 8.5 (OriginLab Corporation, USA). Differences between means were
229 detected using Tukey's test.

230 3. Results and Discussion

231 3.1. Preliminary washing

232 Fig. 2 presents relative electrical conductivity, $Y = \sigma/\sigma^o$, concentration of proteins, Y
233 $= C_p/C_p^o$, and concentration of carbohydrates, $Y = C_c/C_c^o$, versus the number of washing
234 steps, N . Here, the σ^o , C_p^o , and C_c^o correspond to the values for the initial suspension
235 before washing. The relative electrical conductivity, $Y = \sigma/\sigma^o$, continuously decreased
236 with increase of N that corresponds to the dilution of ionic solution in the extracellular
237 aqueous solution. In contrast, the values of $Y = C_p/C_p^o$ (proteins) and $Y = C_c/C_c^o$

238 (carbohydrates) remarkably increased after the first washing step and then decreased.
239 The observed phenomena can be explained by release after first washing a certain
240 amount of water-soluble proteins and carbohydrates initially captured on the surface of
241 algal cells. Obtained data evidenced that application of washing for three times allows
242 significant purification of extracellular solution from water-soluble components.
243 Therefore, in our experiments three washing step of 60 min were applied.

244 3.2. Aqueous extraction

245 In the first step, the aqueous extraction was applied (**Fig. 1a**). The contents of
246 different bio-molecules (electrical conductivity, σ , soluble matter, °Brix, dry weight, DW,
247 contents of carbohydrates, C_c , proteins, C_p , chlorophylls, C_{ch} , and carotenoids, C_{cr}) for
248 the untreated samples (S_1 , without preliminary washing) and (S_3 , with preliminary
249 washing) were compared. All characteristics obtained for the samples S_1 and S_3 were
250 significant different ($p < 0.05$). For example, the content of carbohydrates obtained for
251 the sample S_1 ($C_c = 26.76 \pm 0.81$ mg/g) was ≈ 2 folds higher than those obtained for the
252 sample S_3 ($C_c = 14.32 \pm 1.17$ mg/g). The content of proteins obtained for the sample S_1
253 ($C_p = 24.09 \pm 0.93$ mg/g) was ≈ 39 folds higher than those obtained for the sample S_3
254 ($C_p = 0.61 \pm 0.06$ mg/g). Electrical conductivity, the contents of chlorophylls and
255 carotenoids obtained for the sample S_1 were also significantly higher (approximately
256 one order of magnitude) than those obtained for the sample S_3 ($p < 0.05$). This fact can
257 be explained by the presence of some compounds on the surface of the microalgal cells
258 that can released by procedure of washing.

259 Moreover, the effect of HVED treatment on extraction of different components can
260 be characterized by ratio of different measured values, $R = \text{HVED treated/untreated}$. **Fig.**
261 **3** presents the values of R , (ratios of electrical conductivity, σ ; soluble matter, °Brix; dry
262 weight, DW; contents of carbohydrates, C_c , proteins, C_p , chlorophylls, C_{ch} , and
263 carotenoids, C_{cr}) obtained for unwashed samples ($R = S_2/S_1$) (a) and for washed samples
264 ($R = S_4/S_3$) (b). In all cases, the values of R were higher than 1. It reflects the positive
265 effect of HVED treatment on extraction of intracellular components. For unwashed
266 samples, the maximum ratios were observed for total chlorophylls ($R \approx 1.59 \pm 0.01$),
267 carbohydrates ($R \approx 1.42 \pm 0.03$), proteins ($R \approx 1.36 \pm 0.03$) and carotenoids ($R \approx 1.33 \pm$
268 0.01). For washed samples, the maximum ratios were observed for proteins ($R \approx 30.1 \pm$
269 2.3), carotenoids ($R \approx 22 \pm 1.0$), and total chlorophylls ($R \approx 7.6 \pm 0.2$). The obtained
270 data evidenced the high efficiency of HVED application for recovery of different bio-
271 molecules. It can be explained by the feasibility of HVED to electroporate the cell
272 membrane and induce damage the microalgal cell walls (Grimi et al., 2014). All these
273 findings also confirmed the possibility to attain the selective extraction of different bio-
274 molecules by using the different modes of washing combined with HVED treatment.

275 **3.3.** *Organic solvent extraction*

276 In the second step, the organic solvent extraction was proceeded. To remove water
277 from sediment the vacuum drying was initially performed. **Fig. 4** presents the moisture
278 ratio, MR , and temperature inside centre of the sample, T , versus the drying time, t , for
279 untreated (solid lines, filled symbols) and HVED treated (dashed lines, open symbols)

280 suspensions of unwashed samples (S_1 and S_2) (a) and washed samples (S_3 and S_4) (b).
281 During the VD, the values of MR continuously decreased for all samples (S_1 - S_4), and
282 HVED treatment noticeably accelerated the drying processes. No significant differences
283 in $MR(t)$ were observed for samples without and with washing.

284 Three different stages of the variation of temperatures $T(t)$ during the VD were
285 observed. During the first heating stage, the initial increase of temperature with water
286 evaporation from the surface of slurry was observed. During the drying stage, the
287 intensive evaporation of moisture with the stabilization of the temperature at near
288 constant temperatures level of $T \approx 40$ °C was observed. Finally, at long drying time and
289 relatively low MR below ≈ 0.2 , the reduced drying rate stage with further increase in
290 temperature up to the temperature of VD chamber, $T = 50$ °C, was observed.

291 The evolutions of temperature $T(t)$ were rather different for untreated (samples S_1
292 and S_3) and HVED treated (samples S_2 and S_4) suspensions. Particularly, periods of the
293 near constant temperature ($T \approx 40$ °C) were more clearly displayed for untreated
294 suspensions (they lasted for ≈ 4000 - 7500 s, samples S_1 and S_3), and the reduced drying
295 rate stages were started earlier for HVED treated suspensions (they lasted for ≈ 6000 s
296 for sample S_2 , and for ≈ 5000 s for sample S_4). The HVED treatment accelerated the
297 drying, and the final moisture content of $MR = 0.01$ required VD time of ≈ 16200 s and
298 ≈ 15000 s for untreated (samples S_1 and S_3) and HVED treated (S_2 and S_4 samples)
299 suspensions, respectively. It evidently reflected positive effects of HVED treatment of
300 acceleration of VD process.

301 3.3.1 Extraction of pigments

302 **Fig. 5** presents the examples of extraction kinetics of pigments for unwashed (S_1
303 and S_2) and washed (S_3 and S_4) microalgae with different final values of MR_f after VD
304 ($MR_f = 0.01$ and 0.2). The content of total chlorophylls, C_{ch} , continuously increased with
305 increasing of extraction time, t_e , and no saturation was observed even at relatively long
306 extraction time of $t_e = 28800$ s (8 h). For carotenoids, the near-saturation behaviour was
307 observed for extraction time above $t_e \approx 10000-15000$ s.

308 For HVED pretreated samples, the larger contents of extracted chlorophylls and
309 carotenoids were obtained. For example, for samples with $t_e = 28800$ s and $MR_f = 0.2$,
310 the following values were obtained:

$C_{ch} \approx 0.95$ mg/g DM (unwashed), $C_{ch} \approx 1$ mg/g DM (washed) for HVED treated suspensions;

$C_{ch} \approx 0.71$ mg/g DM (unwashed), $C_{ch} \approx 0.80$ mg/g DM (washed) for untreated suspensions.

311 The washing favored the extraction efficiency, but the differences for unwashed
312 (**Fig. 5a, c**) and washed (**Fig. 5b, d**) were less significant. However, the extraction
313 efficiency of both chlorophylls and carotenoids for less dried samples with $MR_f = 0.2$
314 was significantly higher than for the samples with $MR_f = 0.01$. It evidently reflects the
315 retardation of extraction from overdried samples with less developed porous structure.

316 3.3.2. Extraction of lipids

317 The presence of moisture can hamper the lipids extraction from the microalgae and
318 moisture removal is an important factor to obtain high lipids extraction yield (Bagchi et
319 al., 2015). In our experiments, the extraction of lipids was studied for unwashed

320 (samples S₁ and S₂) and washed (samples S₃ and S₄) microalgae biomass with the final
321 value of $MR_f = 0.01$ after VD. For comparison, the extraction for the initial sample S₀
322 (with washing for 60 min without any treatment) was also studied.

323 **Fig. 6** compares the effects of different procedures performed in this study (Fig. 1)
324 on total lipids content (TLC), C_l , extracted from the microalgae sediment using non-
325 aqueous solvent CHCl₃/MeOH (2:1, v/v). The values of C_l for the samples S₀, S₁ and S₃
326 (without HVED treatment) were approximately the same ($C_l \approx 170 \pm 3.2$ mg/g DM).
327 However, for the samples with HVED treatment (S₂ and S₄), the lipid content increased
328 up to $C_l \approx 200 \pm 2.1$ mg/g DM. The significant effect of HVED treatment ($p < 0.05$) on
329 extraction of lipids can be explained by the breakdown of the microalgal cells.

330 Commonly, the HVED treatment is accompanied with different processes including the
331 electrical breakdown, propagation of streamer, bubble formation and cavitations, light
332 emission, appearing of localized regions with high pressure, and formation of shock and
333 acoustic waves (Boussetta and Vorobiev, 2014). The previous experiments evidenced
334 the presence of strong fragmentation of suspended biocells by HVED treatment (Grimi
335 et al., 2014; Shynkaryk et al., 2009). However, the differences between the unwashed
336 (S₁ or S₂) and washed (S₃ or S₄) samples were insignificant because the small extraction
337 efficiency of lipids in water.

338 The TLC includes different fatty acids such as SFA, MUFA and PUFA. The
339 composition of these lipids directly influences the efficiency of biofuel conversion and
340 its quality, being rich in SFA and MUFA (such as palmitoleic acid and oleic acid) are

341 most favourable for biodiesel production (Nascimento et al., 2013). The relative content
342 of the fatty acids (SFA, MUFA, and PUFA) in extracts were found to be rather similar
343 for different samples S₀-S₄ ($R_{SFA} \approx 27\text{-}32\%$, $R_{MUFA} \approx 33\text{-}39\%$, and $R_{PUFA} \approx 31\text{-}36\%$). It
344 reflects that washing mode and HVED treatment did not affect significantly the
345 composition of fatty acids in extracted lipids.

346 The relative content of different fatty acids methyl ester (FAME) was also
347 determined. A transesterification step was used to obtain FAME content. The palmitic
348 C16:0 (25-29%), palmitoleic C16:1n-7 (28-32%) and eicosapentaenoic C20: 5n-3 (24-
349 31%) acids were predominant in FAME profiles. For palmitic acid C16:0 and
350 palmitoleic acid C16:1n-7, the highest extractions were observed for the samples with
351 HVED treatment (samples S2 and S4). It correlates with data obtained for the TLC (Fig.
352 6). However, for eicosapentaenoic acid C20: 5n-3, the biggest value of the relative
353 content was observed for the sample S1. It reflects that preliminary washing and HVED
354 treatment can selectively affect the content of some FAME in non-aqueous extracts.

355 4. Conclusions

356 Two-step procedure with the initial aqueous extraction from raw microalgae and
357 secondary organic solvent extraction from vacuum dried microalgae were applied for
358 selective recovery of bio-molecules from *N. oculata*. The effects of preliminary washing
359 and HVED pre-treatment were tested. The application of combined washing and HVED
360 treatment significantly enhanced efficiency of aqueous extraction of ionics,
361 carbohydrates, proteins and pigments. Moreover, HVED treatment noticeably

362 accelerated the VD process, and increased extraction yield of chlorophylls, carotenoids
363 and lipids in organic solvent. Partial drying (to 2% of residual moisture content) favored
364 extraction of chlorophylls and carotenoids.

365 **Acknowledgements**

366 Rui Zhang would like to acknowledge the financial support of China Scholarship
367 Council for thesis fellowship. The authors would like to thank Mrs. Delphine Drouin
368 and Mrs. Laurence Lavenant for their technical assistance. The authors would like to
369 thank Mrs. Christa Aoude for editing the English language and grammar of the
370 manuscript.

371 **Appendix. Supplementary data**

372 E-supplementary data of this work can be found in online version of the paper.

373 **Conflict of interest**

374 The authors declare that they have no conflict of interest.

375

376 **References**

- 377 1. Ansari, F.A., Gupta, S.K., Nasr, M., Rawat, I., Bux, F., 2018. Evaluation of various
378 cell drying and disruption techniques for sustainable metabolite extractions from
379 microalgae grown in wastewater: A multivariate approach. *J. Clean. Prod.* 182,
380 634–643.
- 381 2. Bagchi, S.K., Rao, P.S., Mallick, N., 2015. Development of an oven drying protocol
382 to improve biodiesel production for an indigenous chlorophycean microalga
383 *Scenedesmus* sp. *Bioresour. Technol.* 180, 207–213.
- 384 3. Barba, F.J., Grimi, N., Vorobiev, E., 2015. New approaches for the use of non-
385 conventional cell disruption technologies to extract potential food additives and
386 nutraceuticals from microalgae. *Food Eng. Rev.* 7, 45–62.
- 387 4. Boussetta, N., Vorobiev, E., 2014. Extraction of valuable biocompounds assisted by
388 high voltage electrical discharges: a review. *Comptes Rendus Chim.* 17, 197–203.
- 389 5. Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of
390 microgram quantities of protein utilizing the principle of protein-dye binding. *Anal.*
391 *Biochem.* 72, 248–254.
- 392 6. Buchmann, L., Frey, W., Gusbeth, C., Ravaynia, P.S., Mathys, A., 2019. Effect of
393 nanosecond pulsed electric field treatment on cell proliferation of microalgae.
394 *Bioresour. Technol.* 271, 402–408.
- 395 7. Chiu, S.Y., Kao, C.Y., Tsai, M.T., Ong, S.C., Chen, C.H., Lin, C.S., 2009. Lipid

- 396 accumulation and CO₂ utilization of *Nannochloropsis oculata* in response to CO₂
397 aeration. *Bioresour. Technol.* 100, 833–838.
- 398 8. Daneshvar, E., Zarrinmehr, M.J., Hashtjin, A.M., Farhadian, O., Bhatnagar, A., 2018.
399 Versatile applications of freshwater and marine water microalgae in dairy
400 wastewater treatment, lipid extraction and tetracycline biosorption. *Bioresour.*
401 *Technol.* 268, 523–530.
- 402 9. Doan, Y.T.T., Obbard, J.P., 2015. Two-stage cultivation of a *Nannochloropsis* mutant
403 for biodiesel feedstock. *J. Appl. Phycol.* 27, 2203–2208.
- 404 10. Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. t, Smith, F., 1956.
405 Colorimetric method for determination of sugars and related substances. *Anal.*
406 *Chem.* 28, 350–356.
- 407 11. Gerde, J.A., Montalbo-Lomboy, M., Yao, L., Grewell, D., Wang, T., 2012.
408 Evaluation of microalgae cell disruption by ultrasonic treatment. *Bioresour.*
409 *Technol.* 125, 175–181.
- 410 12. Gerken, H.G., Donohoe, B., Knoshaug, E.P., 2013. Enzymatic cell wall degradation
411 of *Chlorellavulgaris* and other microalgae for biofuels production. *Planta* 237,
412 239–253.
- 413 13. Grimi, N., Dubois, A., Marchal, L., Jubeau, S., Lebovka, N.I., Vorobiev, E., 2014.
414 Selective extraction from microalgae *Nannochloropsis* sp. using different methods
415 of cell disruption. *Bioresour. Technol.* 153, 254–259.

- 416 14. Hosseinizand, H., Sokhansanj, S., Lim, C.J., 2018. Studying the drying mechanism
417 of microalgae *Chlorella vulgaris* and the optimum drying temperature to preserve
418 quality characteristics. *Dry. Technol.* 36, 1049–1060.
- 419 15. Liu, C., Grimi, N., Lebovka, N., Vorobiev, E., 2018. Effects of pulsed electric fields
420 treatment on vacuum drying of potato tissue. *LWT* 95, 289–294.
- 421 16. Makkar, H.P.S., Tran, G., Heuze, V., Giger-Reverdin, S., Lessire, M., Lebas, F.,
422 Ankers, P., 2016. Seaweeds for livestock diets: a review. *Anim. Feed Sci. Technol.*
423 212, 1–17.
- 424 17. Mitra, M., Patidar, S.K., Mishra, S., 2015. Integrated process of two stage
425 cultivation of *Nannochloropsis* sp. for nutraceutically valuable eicosapentaenoic
426 acid along with biodiesel. *Bioresour. Technol.* 193, 363–369.
- 427 18. Mittal, R., Raghavarao, K., 2018. Extraction of R-Phycoerythrin from marine
428 macro-algae, *Gelidium pusillum*, employing consortia of enzymes. *Algal Res.* 34,
429 1–11.
- 430 19. Nascimento, I.A., Marques, S.S.I., Cabanelas, I.T.D., Pereira, S.A., Druzian, J.I., de
431 Souza, C.O., Vich, D.V., de Carvalho, G.C., Nascimento, M.A., 2013. Screening
432 microalgae strains for biodiesel production: lipid productivity and estimation of
433 fuel quality based on fatty acids profiles as selective criteria. *Bioenergy Res.* 6, 1–
434 13.
- 435 20. Parniakov, O., Barba, F.J., Grimi, N., Marchal, L., Jubeau, S., Lebovka, N.,

- 436 Vorobiev, E., 2015. Pulsed electric field and pH assisted selective extraction of
437 intracellular components from microalgae *Nannochloropsis*. *Algal Res.* 8, 128–134.
- 438 21. Parniakov, O., Barba, F.J., Grimi, N., Marchal, L., Jubeau, S., Lebovka, N.,
439 Vorobiev, E., 2015a. Pulsed electric field and pH assisted selective extraction of
440 intracellular components from microalgae *nannochloropsis*. *Algal Res.* 8, 128–134.
441 <https://doi.org/10.1016/j.algal.2015.01.014>
- 442 22. Parniakov, O., Barba, F.J., Grimi, N., Marchal, L., Jubeau, S., Lebovka, N.,
443 Vorobiev, E., 2015b. Pulsed electric field assisted extraction of nutritionally
444 valuable compounds from microalgae *Nannochloropsis* spp. using the binary
445 mixture of organic solvents and water. *Innov. Food Sci. Emerg. Technol.* 27, 79–
446 85. <https://doi.org/10.1016/j.ifset.2014.11.002>
- 447 23. Reboloso-Fuentes, M.M., Navarro-Pérez, A., García-Camacho, F., Ramos-Miras,
448 J.J., Guil-Guerrero, J.L., 2001. Biomass nutrient profiles of the microalga
449 *Nannochloropsis*. *J. Agric. Food Chem.* 49, 2966–2972.
- 450 24. Rivera, E.C., Montalescot, V., Viau, M., Drouin, D., Bourseau, P., Frappart, M.,
451 Monteux, C., Couallier, E., 2018. Mechanical cell disruption of *Parachlorella*
452 *kessleri* microalgae: Impact on lipid fraction composition. *Bioresour. Technol.* 256,
453 77–85.
- 454 25. Shynkaryk, M. V, Lebovka, N.I., Lanoisellé, J.-L., Nonus, M., Bedel-Clotour, C.,
455 Vorobiev, E., 2009. Electrically-assisted extraction of bio-products using high
456 pressure disruption of yeast cells (*Saccharomyces cerevisiae*). *J. Food Eng.* 92,

- 457 189–195.
- 458 26. Talebi, A.F., Mohtashami, S.K., Tabatabaei, M., Tohidfar, M., Bagheri, A.,
459 Zeinalabedini, M., Mirzaei, H.H., Mirzajanzadeh, M., Shafaroudi, S.M., Bakhtiari,
460 S., 2013. Fatty acids profiling: a selective criterion for screening microalgae strains
461 for biodiesel production. *Algal Res.* 2, 258–267.
- 462 27. Uribe, E., Vega-Galvez, A., Heredia, V., Pasten, A., Di Scala, K., 2018. An edible
463 red seaweed (*Pyropia orbicularis*): influence of vacuum drying on physicochemical
464 composition, bioactive compounds, antioxidant capacity, and pigments. *J. Appl.*
465 *Phycol.* 30, 673–683.
- 466 28. Van Vooren, G., Le Grand, F., Legrand, J., Cuine, S., Peltier, G., Pruvost, J., 2012.
467 Investigation of fatty acids accumulation in *Nannochloropsis oculata* for biodiesel
468 application. *Bioresour. Technol.* 124, 421–432.
- 469 29. Zhang, R., Grimi, N., Marchal, L., Lebovka, N., Vorobiev, E., 2019. Effect of
470 ultrasonication, high pressure homogenization and their combination on efficiency
471 of extraction of bio-molecules from microalgae *Parachlorella kessleri*. *Algal Res.*
472 40, 101524.
- 473 30. Zhang, R., Grimi, N., Marchal, L., Vorobiev, E., 2018a. Application of high-voltage
474 electrical discharges and high-pressure homogenization for recovery of
475 intracellular compounds from microalgae *Parachlorella kessleri*. *Bioprocess*
476 *Biosyst. Eng.* 1–8.

- 477 31. Zhang, R., Parniakov, O., Grimi, N., Lebovka, N., Marchal, L., Vorobiev, E., 2018b.
478 Emerging techniques for cell disruption and extraction of valuable bio-molecules
479 of microalgae *Nannochloropsis* sp. *Bioprocess Biosyst. Eng.* 1–14.
- 480 32. Zhu, Z., Li, S., He, J., Thirumdas, R., Montesano, D., Barba, F.J., 2018a. Enzyme-
481 assisted extraction of polyphenol from edible lotus (*Nelumbo nucifera*) rhizome
482 knot: Ultra-filtration performance and HPLC-MS2 profile. *Food Res. Int.* 111,
483 291–298.
- 484 33. Zhu, Z., Luo, X., Yin, F., Li, S., He, J., 2018b. Clarification of Jerusalem artichoke
485 extract using ultra-filtration: Effect of membrane pore size and operation
486 conditions. *Food bioprocess Technol.* 11, 864–873.
- 487
488
489

490 **Figure Captions**

491 **Fig. 1.** The schema of the applied extraction procedures (a), and high voltage electrical
492 discharges (HVED) treatment cell applied in the present experiment (b).

493 **Fig. 2.** Relative quantities, Y , ($Y = \sigma/\sigma^o$ for electrical conductivity, $Y = C_p/C_p^o$ for
494 concentration of proteins, and $Y = C_c/C_c^o$ for concentration of carbohydrates) versus the
495 number of washing steps, N . The σ^o , C_p^o , and C_c^o are the initial values before washing.
496 The measurements were done for 1% dry matter (DM) suspensions.

497 **Fig. 3.** The ratios of different measured values, $R =$ high voltage electrical discharges
498 (HVED) treated/untreated, (electrical conductivity, σ ; °Brix, dry weight, DW; contents
499 of carbohydrates, C_c , proteins, C_p , chlorophylls, C_{ch} , and carotenoids, C_{cr}) obtained for
500 unwashed samples ($R = S_2/S_1$) (a) and for washed samples ($R = S_4/S_3$) (b).

501 **Fig. 4.** Moisture ratio, MR , and temperature inside centre of the sample, T , versus the
502 vacuum drying (VD) time, t , for unwashed samples (S_1 and S_2) (a) and washed samples
503 (S_3 and S_4) (b) obtained for untreated (solid lines, filled symbols) and high voltage
504 electrical discharges (HVED) treated (dashed lines, open symbols) suspensions.

505 **Fig. 5.** The content of total chlorophylls, C_{ch} , and carotenoids, C_{cr} , versus the time of
506 EtOH (95%, v/v) extraction, t_e , for unwashed (S_1 and S_2) and washed (S_3 and S_4)
507 samples with different final moisture ratio, MR_f : S_1 and S_2 , $MR_f = 0.01$ (a), S_3 and S_4 ,
508 $MR_f = 0.01$ (b), S_1 and S_2 , $MR_f = 0.2$ (c) and S_3 and S_4 , $MR_f = 0.2$ (d) obtained for
509 untreated (solid lines, filled symbols) and high voltage electrical discharges (HVED)
510 treated (dashed lines, open symbols) suspensions.

511 **Fig. 6.** Total lipids content, C_l , extracted in chloroform/methanol ($\text{CHCl}_3/\text{MeOH}$, 2/1,
512 v/v) for 6 h for different samples S_0 - S_4 obtained for different procedures performed in
513 this study. The microalgae sediment with the final value of moisture ratio, $MR_f = 0.01$
514 after vacuum drying (VD) was used. Different letters within the same column indicate a
515 significant difference ($p < 0.05$) according to Tukey's test.

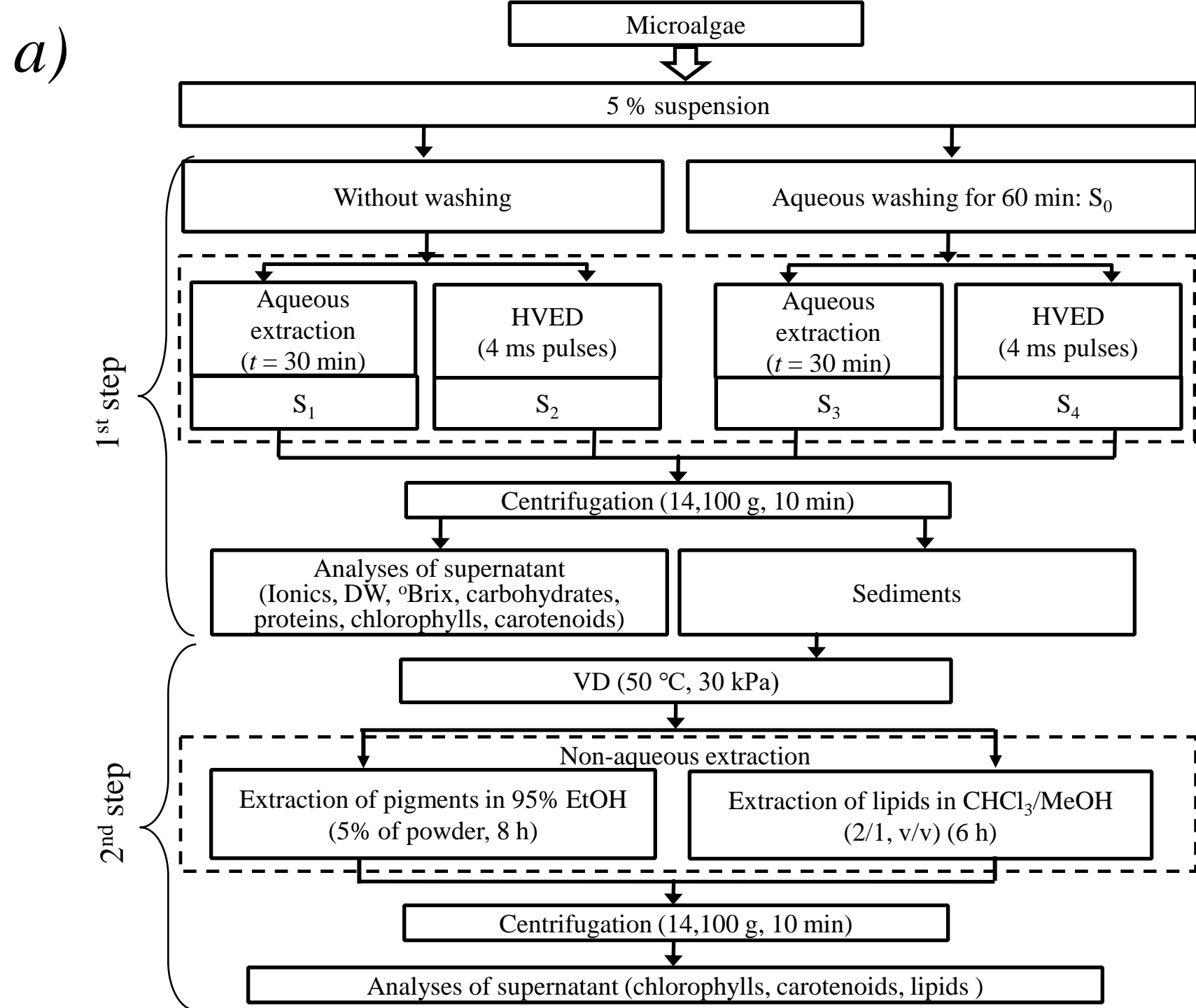
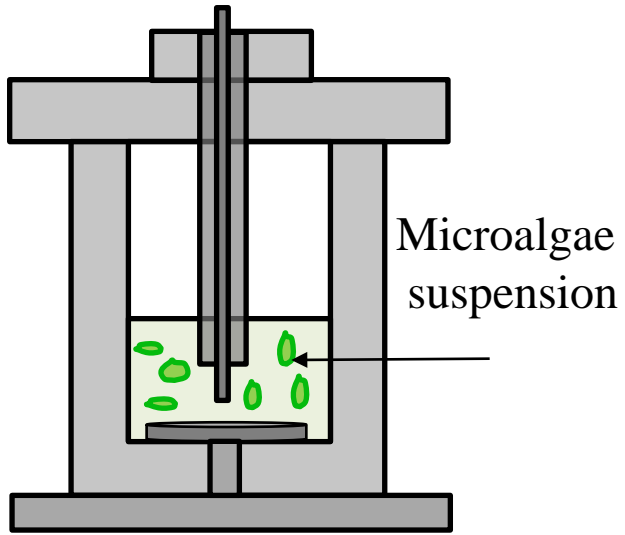


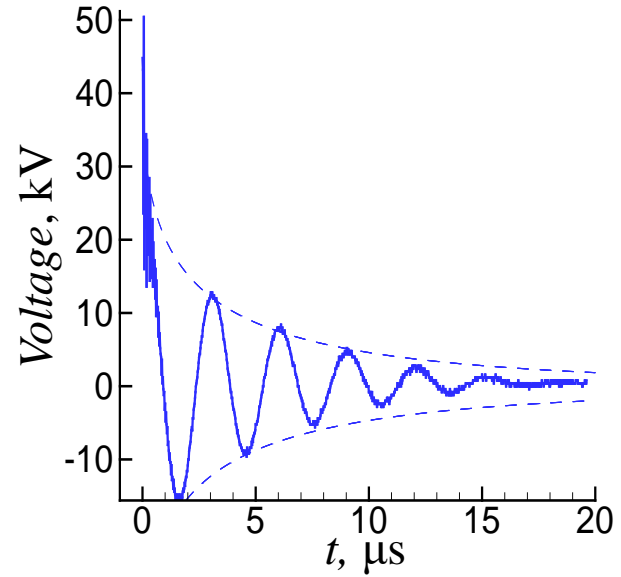
Fig. 1a

b)

Needle to plate geometry



Treatment cell



HVED pulse protocols

Fig. 1b

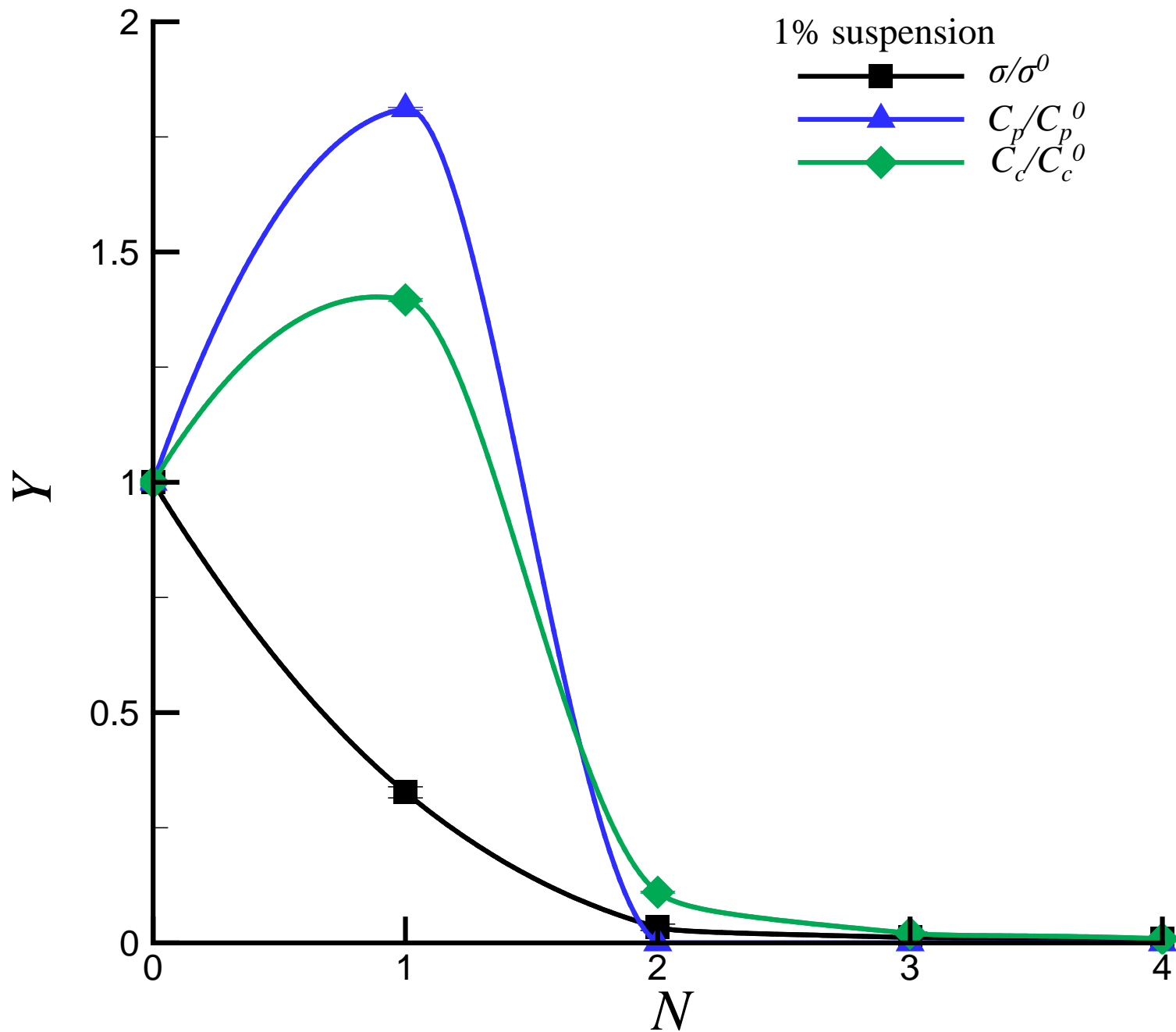


Fig. 2

a)

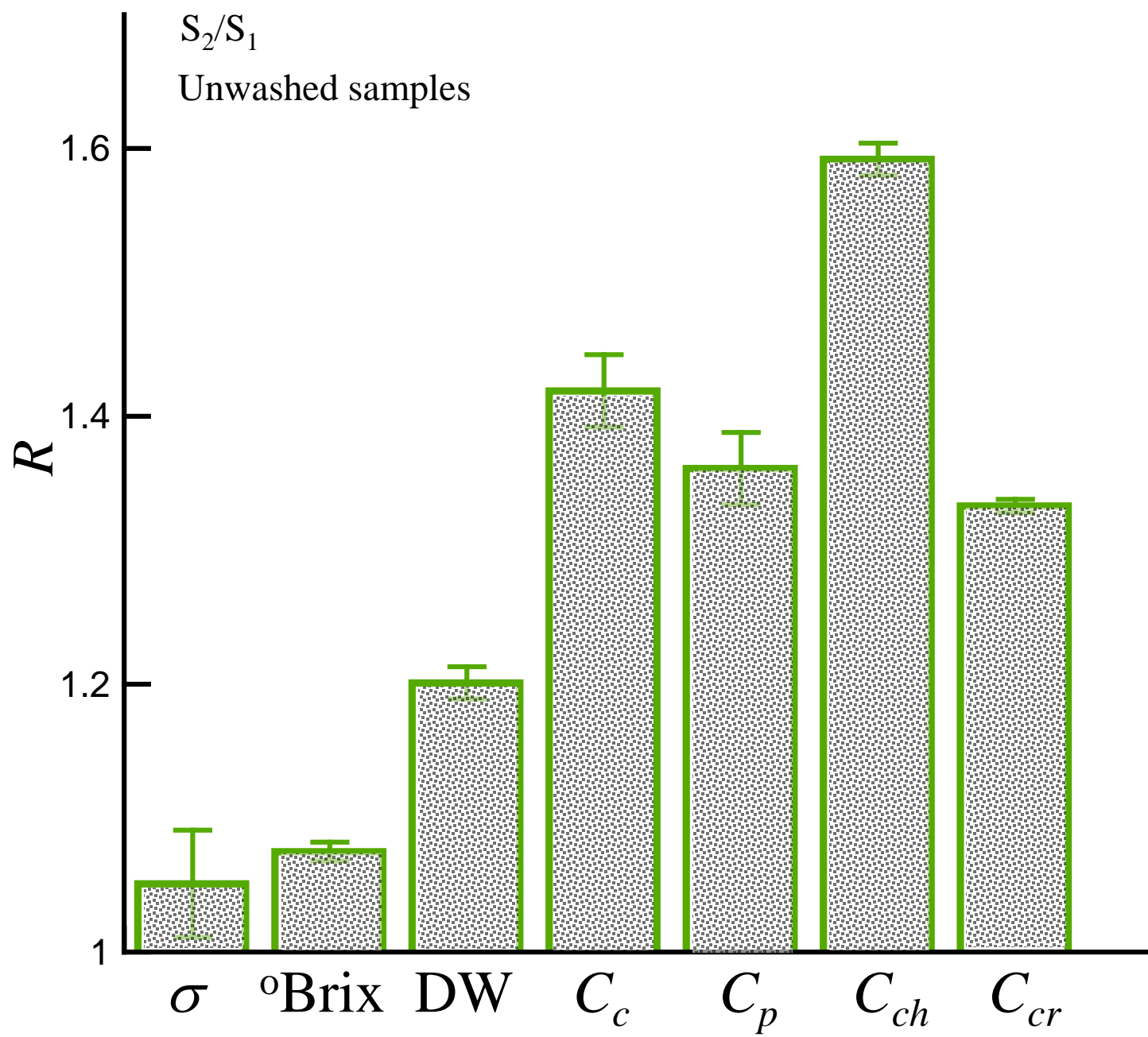


Fig. 3a

b)

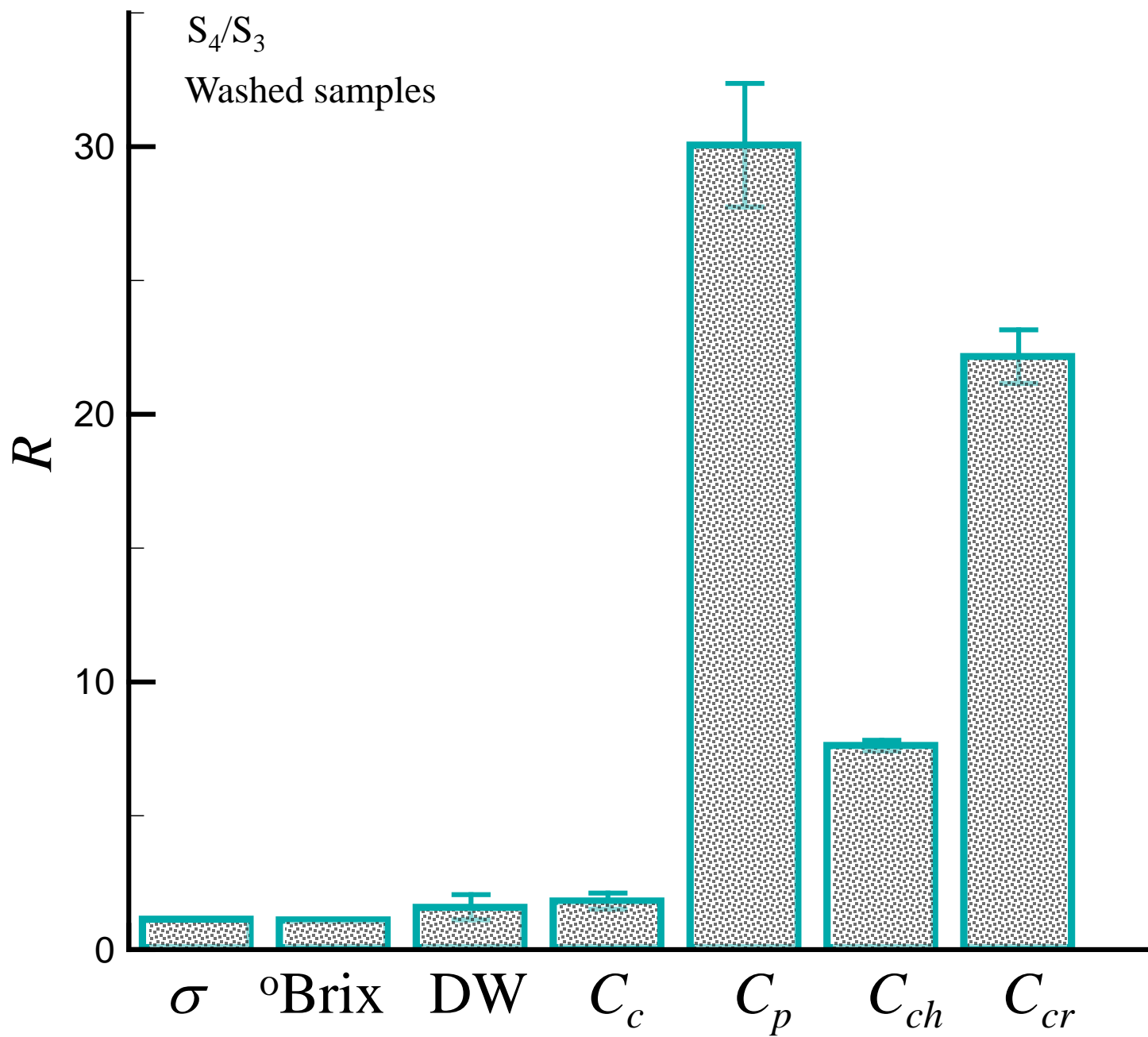


Fig. 3b

a)

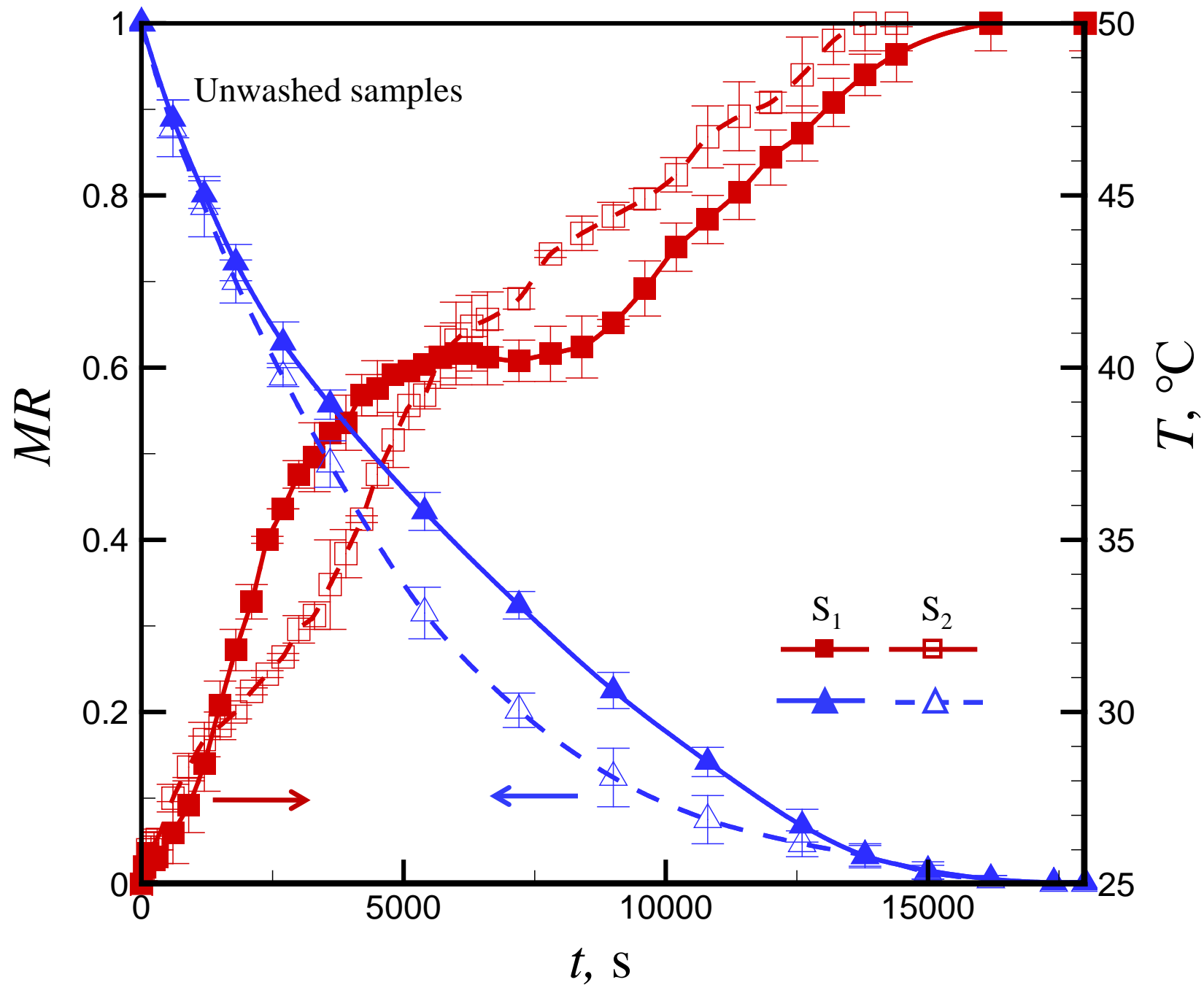


Fig. 4a

b)

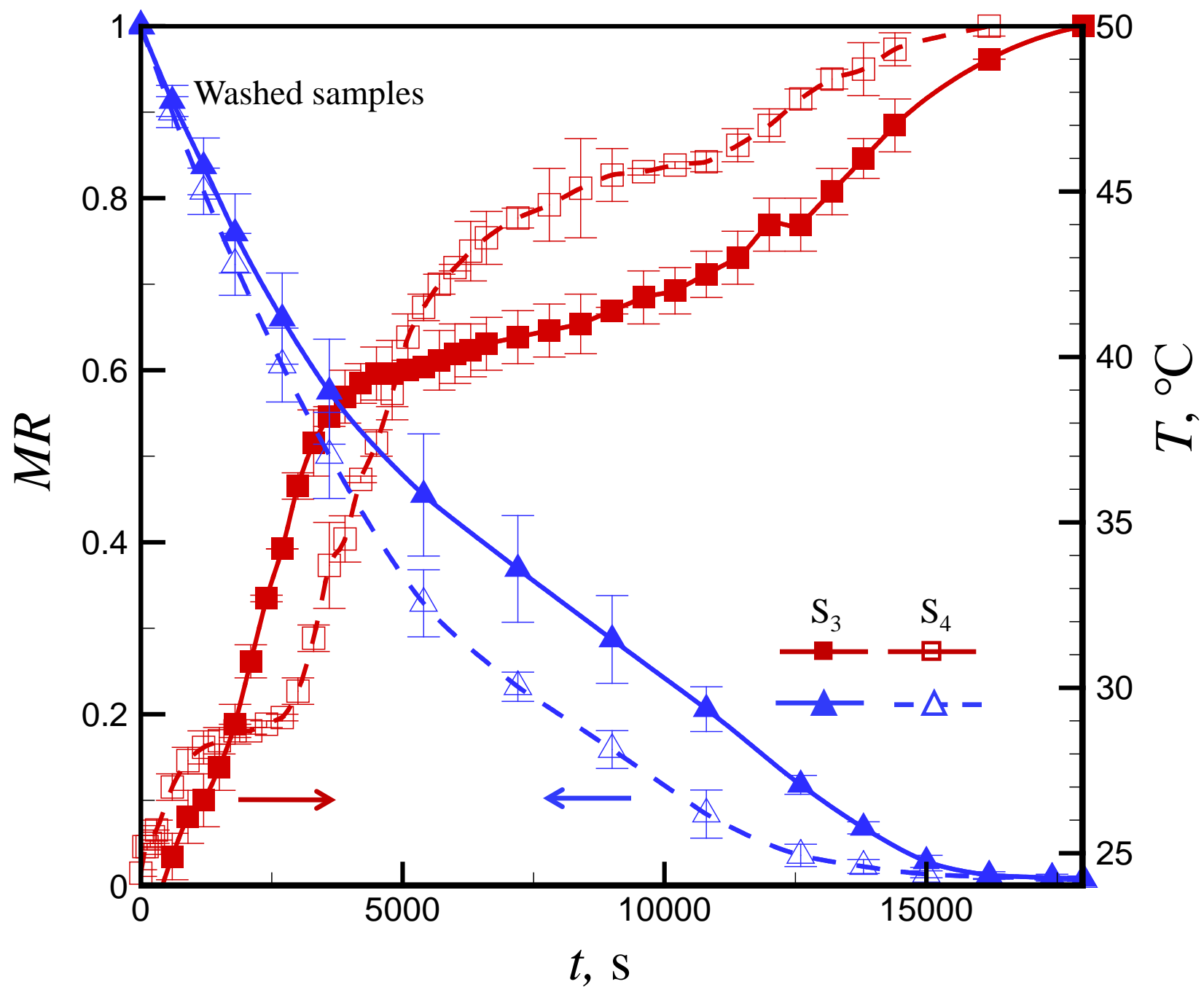


Fig. 4b

a)

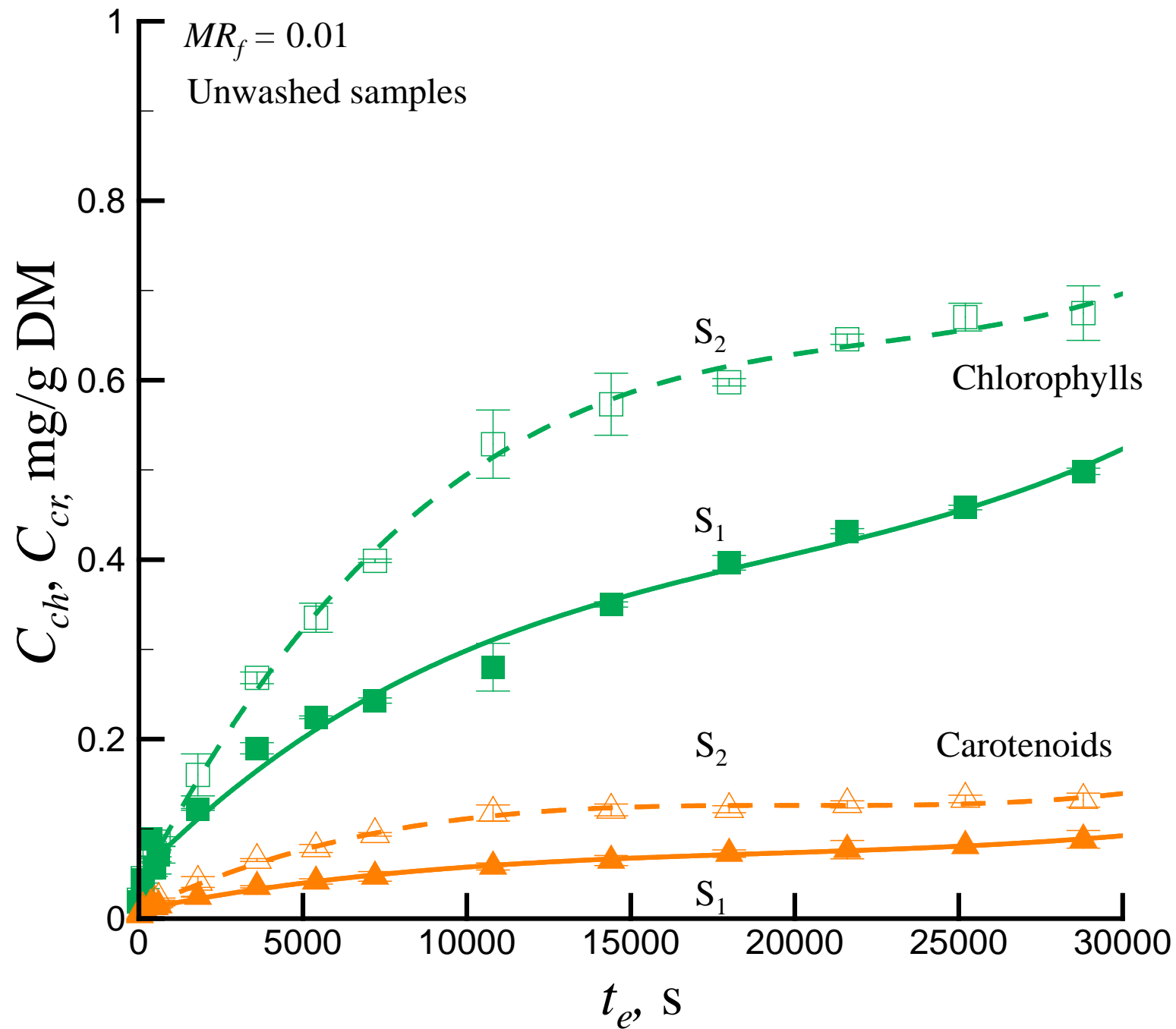


Fig. 5a

b)

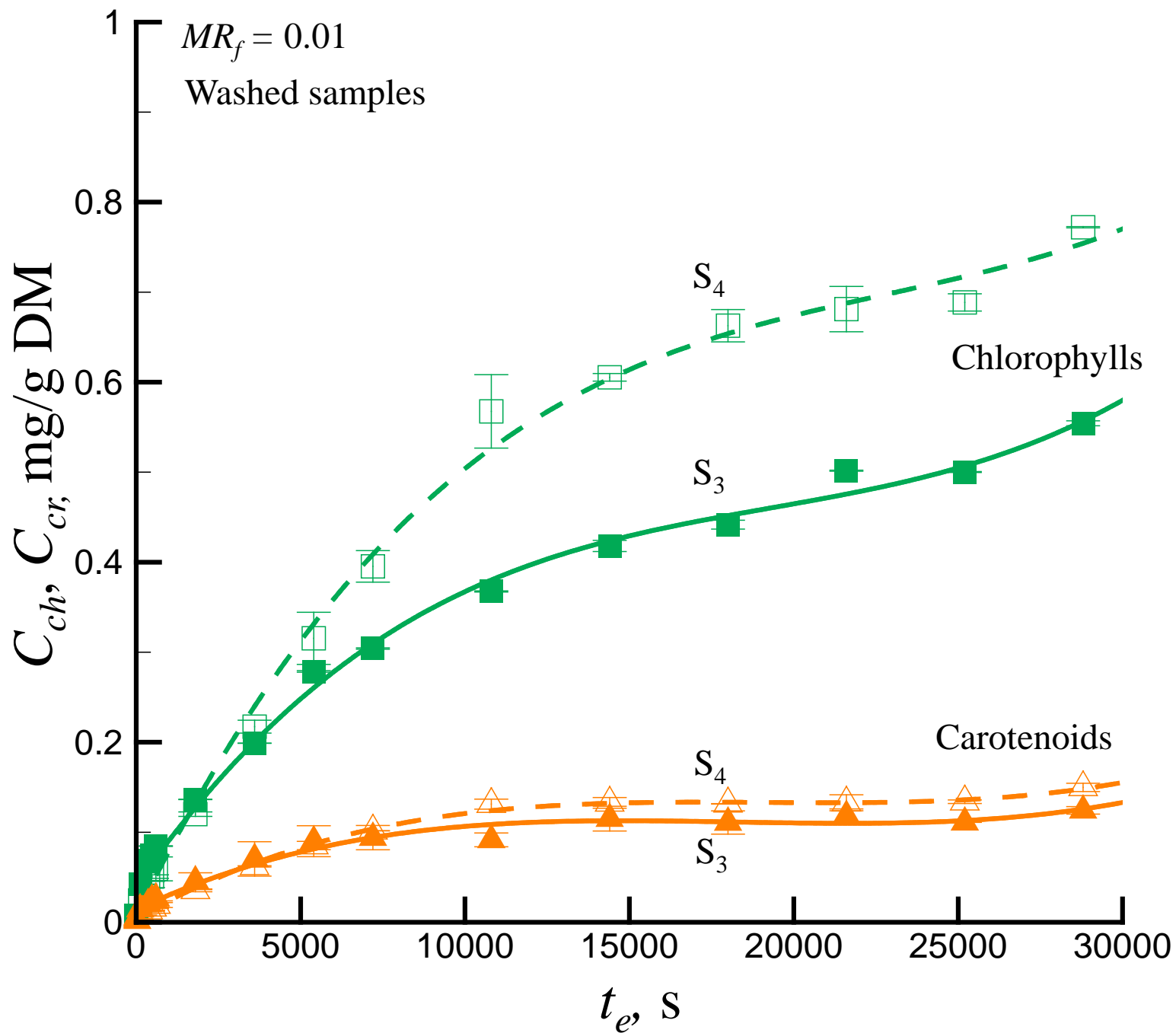


Fig. 5b

c)

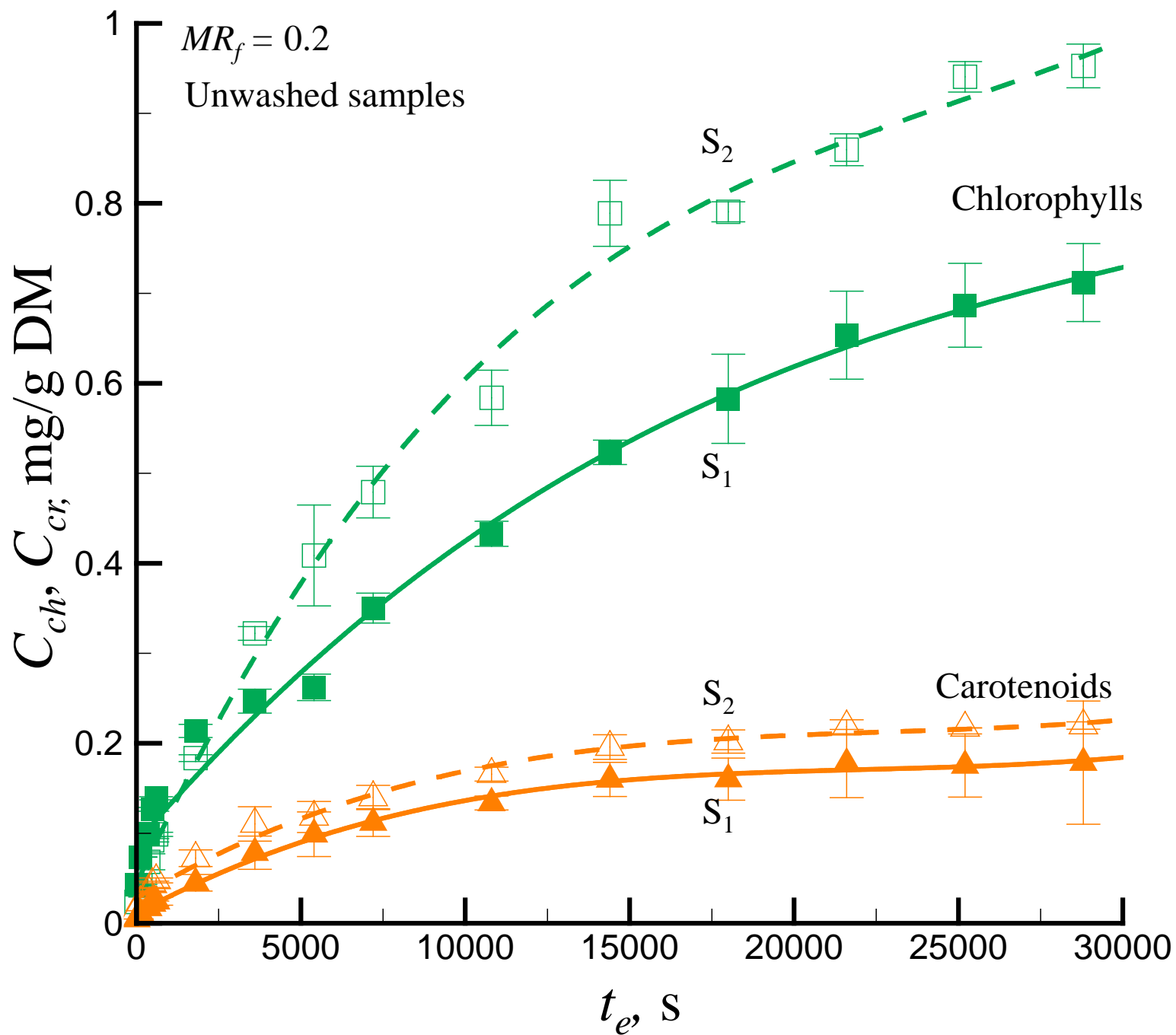


Fig. 5c

d)

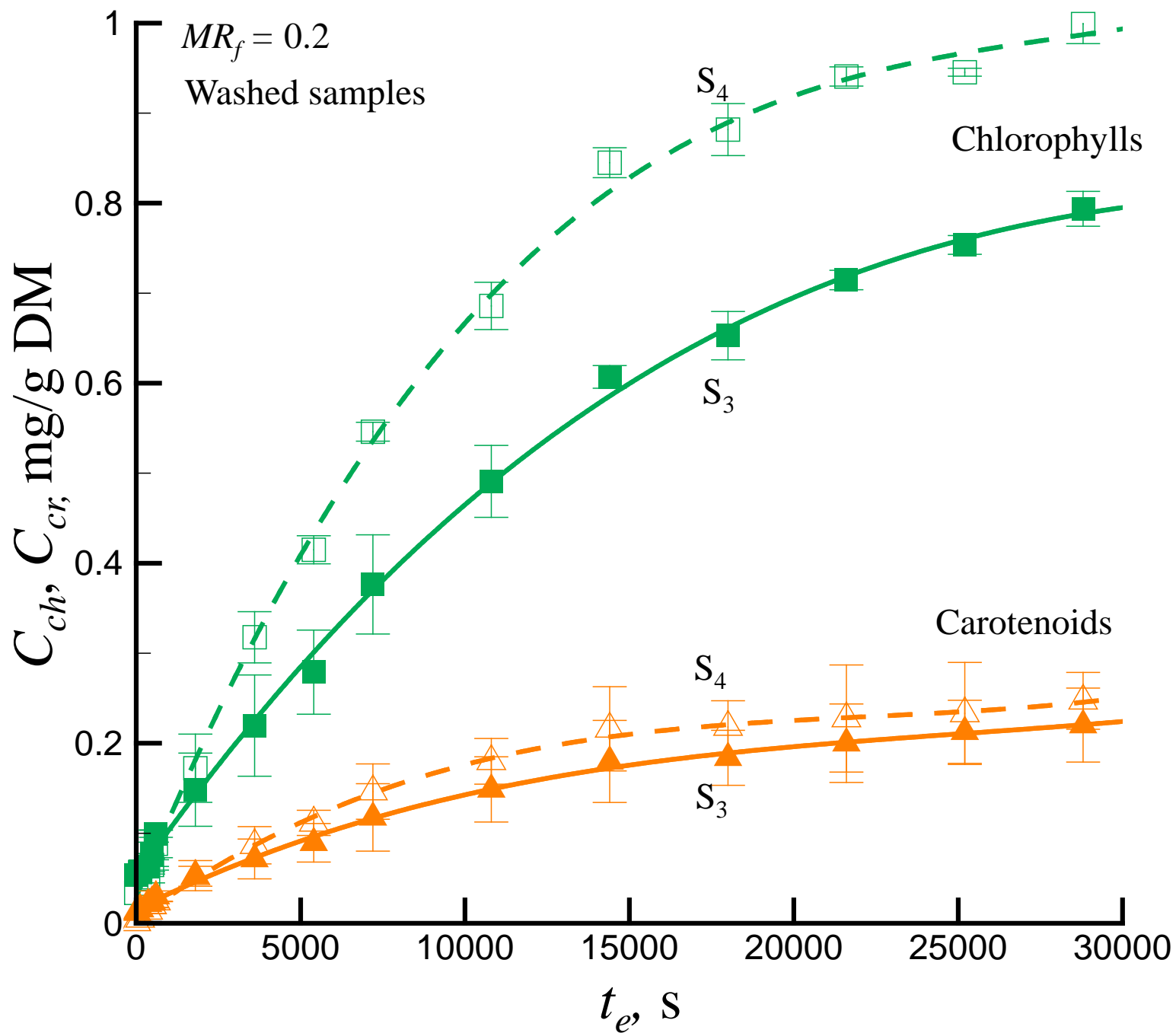


Fig. 5d

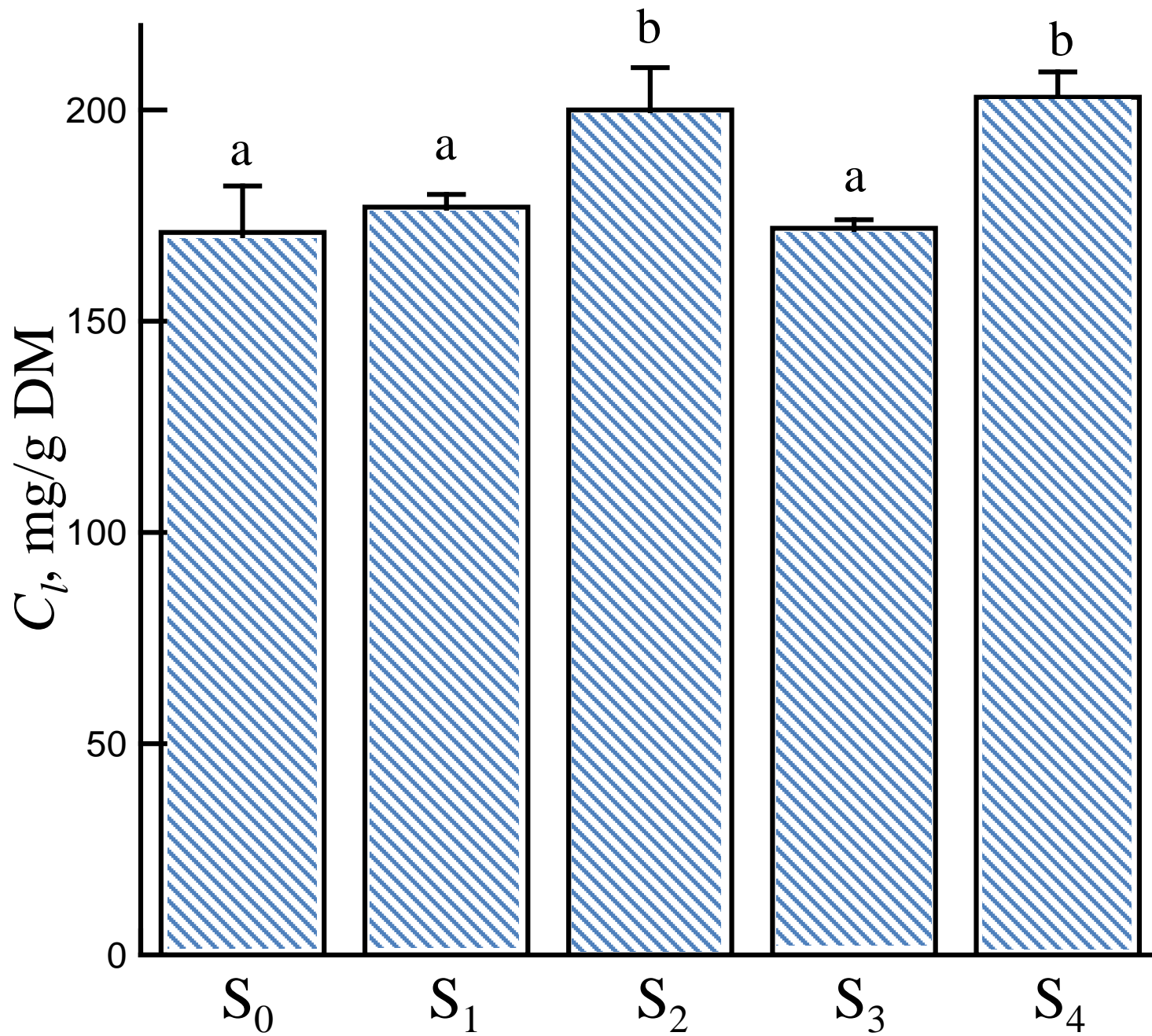


Fig. 6

