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Pulsed electric field-assisted fermentation of *Hanseniaspora* sp. yeast isolated from Lebanese apples

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1 **Abstract**

2 *Hanseniaspora* sp. yeast was stimulated using pulsed electric field (PEF) during the different
3 fermentation phases. The impact of PEF parameters on the growth rate and substrate
4 consumption was studied. The PEF intensities chosen for this study were mainly in the range
5 of 72-285 V.cm⁻¹. A PEF treatment chamber was designed for this study with a ratio of 1:50
6 between the volume of the fermenter and the volume of the chamber. It allows the recycling
7 of the culture medium using a peristaltic pump, and the yeast treatment by PEF during the
8 fermentation. The continuous circulation of the medium allows avoiding the increase of the
9 temperature inside the fermenter, the cell aggregation, as well as the agitation and the scale-
10 up issues that are associated with the PEF treatment of the entire volume in batch mode. The
11 maximal yeast growth rate was obtained using an electric field strength of 285 V.cm⁻¹ applied
12 during both Lag and early exponential phase, and Log phase. This observation was
13 accompanied by a faster consumption of glucose in the medium during the fermentation.
14 Besides, the sensitivity of *Hanseniaspora* sp. yeast to PEF treatment was more pronounced
15 during the Lag and early exponential phase than the Log phase. The results obtained exposed
16 the great benefit of stimulating *Hanseniaspora* sp. yeast using moderate PEF as it reduces the
17 fermentation time along with increasing the biomass concentration.

18

19 **Keywords:** *Hanseniaspora* sp.; fermentation; stimulation; pulsed electric field

20 **1. Introduction**

21 The responses of living organisms under electric field have been attracting great attention
22 for several decades (Berg, 1995; Hart & Palisano, 2017). These responses include changes in
23 the synthesis of DNA and proteins, cell growth, electroporation of the cell membranes,
24 biochemical reactions, electrofusion of cells, and the inactivation or stimulation of
25 microorganisms (Góral & Pankiewicz, 2017; Liu, Lebovka, & Vorobiev, 2013; Martínez,
26 Cebrián, Álvarez, & Raso, 2016; Martínez, Delso, Maza, Álvarez, & Raso, 2019; Teissié et
27 al., 2002). These effects depend on different factors, including the cell type and size, the
28 application time, and the intensity of the electric field. However, the mechanisms behind
29 these changes are complex and not yet fully understood. Nonetheless, multiple industrial
30 applications have been developed specifically for the inactivation of microorganisms (Barba,
31 Koubaa, do Prado-Silva, Orlien, & de Souza Sant'Ana, 2017; El Zakhem, Lanoisellé,
32 Lebovka, Nonus, & Vorobiev, 2006; González-Arenzana et al., 2018; Montanari et al., 2019).
33 This involves the use of high electric field intensity, where the irreversible cell membrane
34 electroporation has been mainly attributed to the inactivation mechanism.

35 When applying low electric field intensities, considered at sub-lethal levels, the
36 microorganisms are stimulated rather than inactivated (El Zakhem et al. 2006; Mattar et al.
37 2014, 2015; Mota et al. 2018; Wang et al. 2018). The first study describing the stimulation of
38 microorganisms in presence of electric field was performed by Heinz Doevenspeck in 1962
39 (cited by Sitzmann, (2016)), who reported that the cultures of *Escherichia coli* being treated
40 with field strengths below $3 \text{ kV}\cdot\text{cm}^{-1}$ seemed to increase their growth rates. This impact of
41 electrostimulation has been patented a few years later (Doevenspeck, 1966). Since that,
42 several examples have been described in the literature concerning the stimulation of
43 microorganisms by PEF (Ohba, Uemura, & Nabetani, 2016; Yeo & Liong, 2013; Yeo, Ong,
44 & Liong, 2014). Through these works, several observations such as the acceleration of

45 fermentation, better production of the metabolites of interest, and a larger number of cells
46 have been reported compared to the non-stimulated controls. For instance, the stimulation of
47 *Saccharomyces cerevisiae* yeast by PEF was successfully carried out in batch mode (Mattar
48 et al. 2014, 2015). In these works, pulses of 20-2000 V.cm⁻¹ for 10⁻⁵ - 1 s (Mattar et al. 2014),
49 and 100 and 6000 V.cm⁻¹ for 100 μs (Mattar et al. 2015) were applied. The stimulation
50 increased in the fermentation rate, mainly in the initial fermentation phase (adaptation phase).
51 Therefore, the results indicate a great potential for the practical implementation of PEF-
52 assisted fermentation technology to improve yeast performances. However, the mechanisms
53 remain yet unclear. There are two possible mechanisms for improved yeast performance by
54 PEF that have been described in the literature, the first one being related to the expression of
55 oxidative stress response genes during PEF treatment (Tanino, Sato, Oshige, & Ohshima,
56 2012), and the second is related to the changes in the activity of certain membrane proteins
57 allowing the assimilation of a larger amount of substrates (Vassu et al., 2004).

58 Regarding the great potential behind implementing PEF technology in the fermentation
59 processes, more investigations are required in this field to further study the impact of low-
60 intensity PEF treatments on microorganisms during the fermentation process and not only
61 prior to it. Most of the studies, above-mentioned, were focused on applying a PEF treatment
62 shortly before the inoculation, and not during the fermentation process itself. This work
63 aimed to evaluate the impact of PEF treatment during the fermentation process and by growth
64 phase. Treating the culture medium in batch mode directly in the fermenter is associated with
65 many issues including the increase of temperature inside the fermenter, the difficulty of
66 agitation, the cell aggregation, as well as the scaling-up problems. Furthermore, studying the
67 impact of PEF during the growth phases is crucial for a better understanding of the microbial
68 stimulation mechanisms. In this line, this paper aimed to study the impact of the continuous
69 PEF treatment on the growth of the non-*Saccharomyces* yeast cells *Hanseniaspora* sp. The

70 growth and glucose consumption kinetics were recorded during the fermentation processes,
71 depending on the electric field strengths and the growth phases. In fact, the genus
72 *Hanseniaspora* was identified during spontaneous fermentation of apple juice (Pando
73 Bedriñana, Querol Simón, & Suárez Valles, 2010; Valles, Bedriñana, Tascón, Simón, &
74 Madrera, 2007), and its positive effects in alcoholic fermentation and cider production has
75 been exposed (Capozzi et al., 2019). It has been reported that the use of this strain may
76 contribute to a better aroma profile of the fermented apple juice (de Arruda Moura
77 Pietrowski, dos Santos, Sauer, Wosiacki, & Nogueira, 2012).

78 **2. Materials and methods**

79 **2.1. Yeast growth**

80 *Hanseniaspora* sp. yeast strain used in this work is belonging to the authors' laboratory
81 strains collection (Al Daccache, Salameh, Maroun, & Louka, 2017). It was previously
82 isolated and identified from spontaneous fermentation of a Lebanese apple juice obtained
83 from the variety "Ace Spur". A Yeast-Extract- Peptone- Dextrose (YPD) medium (1% yeast
84 extract, 2% peptone, 2% dextrose) was used for the culture of *Hanseniaspora* sp. yeast cells.

85 **2.2. Fermentation processes**

86 *2.2.1. Hanseniaspora* sp. pre-culture

87 The pre-cultures for the different fermentations (i.e. control and PEF-assisted) were
88 performed by taking one colony of the yeast strain from a YPD agar plate (1 % yeast extract,
89 2 % peptone, 2 % dextrose, and 2 % agar) into 250 mL YPD liquid medium (1 % yeast
90 extract, 2 % peptone, 2 % dextrose), under sterile conditions (20 min at 121 °C in an
91 autoclave HMC HV-110L (HMC Europe GmbH, Germany)). The incubation was performed
92 during 24 h, at 30 °C temperature, and 250 rpm agitation speed in a shaker (Thermo
93 Scientific, France), which allows the yeast to reach the late exponential phase.

94 2.2.2. *Control fermentations*

95 A volume of 1.5 L of YPD medium (1 % yeast extract, 2 % peptone, 2 % dextrose) was
96 introduced into a 2-L fermenter (LSL Biolafitte S.A.). After sterilization as above-mentioned
97 and cooling to 30 °C, the medium was inoculated by the pre-culture to get an initial
98 concentration of 4.5×10^6 cells.mL⁻¹, corresponding to an initial OD of 0.1. The control
99 fermentation in batch mode without medium circulation (Figure 1.A) was performed at 250
100 rpm agitation speed, and temperature maintained at 30 °C. After recording the kinetics of
101 growth and glucose consumption in batch mode without medium circulation, a treatment
102 chamber was designed to allow the circulation of the medium and the yeast treatment by PEF
103 during the fermentation (Figure 1.B). The medium circulation is required since the treatment
104 for a long time during the fermentation may induce the cell aggregation (El Zakhem et al.,
105 2006), and an undesirable increase in the medium temperature (Morales-de la Peña, Elez-
106 Martínez, & Martín-Belloso, 2011). For this purpose, two parallel stainless steel electrodes (3
107 mm width, 6 cm diameter) were used and fixed between two insulators (Figure 1.C and 1.D).
108 A disk-shaped Teflon ring was placed between the electrodes, forming a channel for the
109 streaming liquid and providing a distance between the electrodes of 1.4 cm. The volume of
110 the treatment chamber was thereby around 40 mL (a ratio of 1:50 between the volume of the
111 fermenter and the volume of the chamber). Two pipe fitting connectors were added to make it
112 operate as a continuous chamber. A peristaltic pump allowed the medium circulation from the
113 reactor to the treatment chamber and back into the reactor. Different circulation flow rates
114 were tested (25, 50, and 75 mL.min⁻¹) without PEF application, to get the same growth and
115 glucose consumption kinetics obtained in batch mode without circulation (Figure 1.A). The
116 total length of the tubes used was equal to 90 cm. the treatment chamber and the tubes were
117 sterilized as above-mentioned.

118

119 2.2.3. PEF-assisted fermentations

120 The designed PEF-treatment chamber was used for the electrostimulation of
121 *Hanseniaspora* sp. yeast. For this purpose, the two electrodes were connected to a PEF
122 generator made by the “Service électronique” of the University of Technology of Compiègne
123 (France) (Figure 2.A). It provided bipolar pulses of nearly rectangular shape, with the
124 respective maximal delivered voltage and current of 400 V and 40 A. Three PEF-assisted
125 fermentations were investigated: 1) PEF-treatment for 6 h during the Lag and Log phases, 2)
126 PEF-treatment for 3 h during the Lag (adaptation) and early exponential phase, and 3) PEF-
127 treatment for 3 h during the Log (exponential) phase. The PEF treatments consisted of
128 applying a succession of trains, each composed of $n = 10$ pulses during the Lag and Log
129 fermentation phases. Each pulse duration was fixed to $t_i = 100 \mu\text{s}$, with a pulse period time of
130 $\Delta t = 1 \text{ ms}$. The time between the trains was fixed to $\Delta t = 1 \text{ s}$ (Figure 2.B). When treating the
131 culture medium during the Lag and Log phases corresponding both to 6 h, the number of
132 trains N applied was 10 716, which corresponded to an effective electric treatment t_{PEF} of
133 10.716 s ($t_{PEF} \text{ (s)} = N \cdot n \cdot t_i \text{ (s)}$). Different electric field strengths E were tested in the range
134 of 72-285 $\text{V}\cdot\text{cm}^{-1}$ (Figure 2). For instance, when applying the maximal voltage of 400 V
135 delivered by PEF equipment and taking into account the distance of 1.4 cm between the
136 electrodes, this corresponded to applying 285 $\text{V}\cdot\text{cm}^{-1}$ field intensity. All the output data (e.g.,
137 current I (A) and voltage U (V)) were recorded using a data logger and software developed
138 by the “Service électronique” of the University of Technology of Compiègne (France). The
139 specific power consumption of PEF treatment W ($\text{J}\cdot\text{mL}^{-1}$) was calculated according to
140 Equation (1):

141
$$W \text{ (J}\cdot\text{mL}^{-1}\text{)} = \frac{U \cdot I \cdot t_{PEF}}{V} \quad \text{Equation (1)}$$

142 where U is the voltage (V), I is the current intensity (A), t_{PEF} is the time of the PEF treatment,
143 $t_{PEF} = N \cdot n \cdot t_i$, N is the number of trains, n is the number of pulses per train, and V is the
144 volume of the culture medium in the bioreactor (mL).

145 **2.3. Determination of growth and glucose consumption kinetics**

146 The kinetics of growth and glucose consumption were recorded during the batch
147 fermentation modes with and without culture medium circulation, and during the PEF-
148 assisted fermentations.

149 *2.3.1. Determination of the growth kinetics*

150 The growth kinetics were followed by both 1) measuring the optical density (OD) at 600
151 nm, and 2) determining the cell concentration (cells.mL⁻¹). Samples were taken periodically
152 during the fermentation processes, and their OD values were determined using a Thermo
153 Spectronic BioMate 3 UV-visible spectrophotometer (Thermo Scientific, France). For cell
154 counting, serial dilutions were made for each sample, and the cells were counted using a
155 Thoma cell counting chamber (Preciss, France). To detect the viable cells, the samples were
156 mixed volume to volume with a solution of 0.01 % methylene blue. The biomass
157 concentration X (cells.mL⁻¹) and biomass yield $Y_{X/S}$ (g_{biomass}.g⁻¹_{substrate}) were calculated
158 respectively according to equations (2) and (3),

$$159 \quad X \text{ (cells.mL}^{-1}\text{)} = \frac{a \cdot 250000 \cdot d}{b} \quad \text{Equation (2)}$$

160 where a is the number of cells counted, d is the dilution factor, and b is the number of squares
161 counted in the chamber.

$$162 \quad Y_{X/S} \text{ (g}_{\text{biomass}} \cdot \text{g}_{\text{substrate}}^{-1}\text{)} = \frac{X_f - X_0}{S_0 - S_f} \quad \text{Equation (3)}$$

163 where X_0 and X_f are respectively the initial and the final biomass concentrations (g.L^{-1}), and
164 S_0 and S_f represent respectively the initial and the final glucose concentrations (g.L^{-1}).
165 The maximal specific growth rate μ_{max} (h^{-1}) was determined by plotting $\text{Ln } X$ (cells.mL^{-1})
166 *versus* time (h). μ_{max} (h^{-1}) values correspond to the slopes of the curves obtained during the
167 exponential phases.

168 *2.3.2. Determination of the glucose consumption kinetics*

169 The content of soluble compounds was followed by measuring the °Brix using a digital
170 refractometer (Anggur DR401, France). The concentrations of glucose were determined using
171 high-performance liquid chromatography instrument LC-20AD (Shimadzu, Japan). The
172 samples taken during the fermentation processes (used for the determination of the growth
173 kinetics) were diluted 10 times in ultrapure water, and 1 μL was injected. The separation was
174 performed in isocratic mode (a mixture of 1:4 (20:80, v:v) water/acetonitrile with a flow rate
175 of 3 mL.min^{-1}), and using a Luna 5 μm NH_2 (250 x 4.6 mm) column. The separation
176 temperature was set to 40 °C, and the molecules were detected using a low-temperature
177 evaporative light scattering detector ELSD-LT II (Shimadzu, Japan). The compounds'
178 assignation and quantification were performed using pre-established standard curves of
179 glucose (5-30 g.L^{-1}).

180 The glucose consumption rate was calculated according to equation (4).

$$181 \quad \frac{dS}{dt} \left(\text{g}_{glucose} \cdot \text{L}^{-1} \cdot \text{h}^{-1} \right) = \frac{S_0 - S_f}{t_f - t_0} \quad \text{Equation (4)}$$

182 where S_0 and S_f are respectively the initial and the final substrate concentrations (g.L^{-1}),
183 whereas t_0 and t_f are respectively the initial and the final times.

184 **2.4. Scanning electron microscopy**

185 The impact of PEF on *Hanseniaspora* sp. yeasts was followed using an ESEM Quanta
186 FEG 250 Scanning Electron Microscope (Philips, USA). The pictures were taken after 6 h
187 fermentation, for the untreated and the PEF-treated culture at 285 V.cm^{-1} , respectively. The
188 samples (1 mL) taken from the fermenter were immediately centrifuged at 13000 g for 2 min,
189 and the yeast pellet was washed twice with 1 mL physiologic water and centrifuged under the
190 same conditions to remove the medium traces. Each sample was then resuspended in
191 physiologic water, and $\approx 20 \text{ }\mu\text{L}$ were placed on the analysis plate of the microscope. A
192 vacuum-drying step was then performed at $5 \text{ }^\circ\text{C}$ and 800 Pa ($\approx 6 \text{ Torr}$), and the samples were
193 analyzed by electron beam at 20 kV. All pictures were taken at the physicochemical analysis
194 service of the University of Technology of Compiègne (France).

195 **2.5. Statistical analyses**

196 Each fermentation experiment was repeated three times. One-way analysis of variance
197 (ANOVA) was used for the statistical analysis of the data with the help of Statgraphics Plus
198 software (version 5.1, Statpoint Technologies Inc., Warrenton, VA, USA). The significance
199 level at 95% confidence was taken for each analysis. The values presented in the figures
200 correspond to the mean value of the repetition, and the error bars correspond to the standard
201 deviations of each measurement.

202 **3. Results and discussion**

203 A medium circulation flow rate of 75 mL.min^{-1} allowed obtaining the same growth
204 kinetic compared to the control fermentation without circulation. It was thereby used for
205 PEF-assisted fermentations. First, PEF was applied between the electrodes of the treatment
206 chamber during the Lag (adaptation) and the Log (exponential) phases, corresponding
207 together to 6 h treatment, and an effective PEF application time of 10.716 s. The results
208 obtained and presented in figure 3 show an increase in the yeast concentrations in the

209 fermenter, proportionally to the PEF intensity, compared to the untreated suspension.
210 Applying PEF intensities of 72, 143, and 215 V.cm⁻¹ seems to induce a nearly similar
211 stimulation effect on yeasts, with no significant differences between the three growth
212 kinetics. The stimulation effect was more pronounced when an intensity of 285 V.cm⁻¹ was
213 applied (Figure 3.A). In fact, it has been previously reported that the electrostimulation is
214 energy (J.mL⁻¹) dependent, and it is not directly related to the intensity of the electric field E
215 applied (Mattar et al. 2014). Treating the cell suspension at 285 V.cm⁻¹ leads to a significant
216 increase in the final biomass concentration by 75 %, corresponding to an increase in the
217 number of cells from 9.0*10⁷ (for the control experiment) to 1.6*10⁸ cells.mL⁻¹. These results
218 were supported by the measurements of the optical densities (OD values at 600 nm), which
219 demonstrated a significant increase (from 2.43 for the control experiment to 3.23 for the PEF-
220 assisted fermentation) at 285 V.cm⁻¹, and after 8 h of fermentation (Figure 3.B). Besides, the
221 highest specific growth rate μ_{max} (0.47 h⁻¹) was observed for the PEF-assisted fermentation at
222 285 V.cm⁻¹ (Table 1), which indicates a faster division of cells, involving a faster cell growth.

223 Applying 285 V.cm⁻¹ field intensity to the cell suspension allowed a significant
224 shortening of the Lag phase by around 1 h, compared to the untreated suspension (Lag phase
225 \approx 1.5 h). This behavior was previously observed by Castro et al., (2005), who reported similar
226 results when treating a recombinant *S. cerevisiae* yeast (NCYC869-A3/pVK1.1) at low
227 electric field intensities. Their results showed a greater biomass concentration of almost 3
228 times compared to the control, and a shorter Lag phase for the treated suspensions. Similarly,
229 *S. cerevisiae* yeasts were stimulated during the grape fermentation when treated with PEF,
230 showing an increase in biomass growth and sugar depletion (Mattar et al. 2013). In another
231 study, the influence of static magnetic fields on *S. cerevisiae* yeast has been demonstrated,
232 showing that the rate of the biomass growth in the samples of the magnetized cultures was
233 3.7 times higher than that of the control cultures (Muniz, Marcelino, Motta, Schuler, &

234 Motta, 2007). A 100% increase in *S. cerevisiae* growth was also shown under pulses of 0.85
235 kV.cm⁻¹ (Fologea, Vassu-Dimov, Stoica, Csutak, & Radu, 1998). A possible explanation of
236 these observations could be an increase in the yeast cells' division during the PEF treatment,
237 which leads to a higher biomass concentration. In fact, it was reported that the use of PEF
238 could induce some DNA damages, which affects the mitosis checkpoints and the cell cycle
239 phase distribution, compared to the non-treated cells (Stacey et al., 2003). Kerns, Bauer, &
240 Berg, (1993) showed that the PEF-induced membrane permeability caused the growth
241 stimulation of *Trichoderma reesei* at 1.5 mV.cm⁻¹ pulses.

242 The results of the growth kinetics were supported by the quantification of glucose
243 consumption during the fermentations. Figure 4 presents both the evolution of °Brix and the
244 glucose concentrations (determined by HPLC) for the PEF-assisted fermentations, compared
245 to the control. The results show that the °Brix values concur with that of the glucose
246 concentrations. Both values decreased faster for the PEF-treated samples, compared to the
247 control fermentation, with the fastest glucose depletion obtained for the PEF-treatment
248 applied at 285 V.cm⁻¹. After 8 h of fermentation, the °Brix decreased from 5.9% (g.100 mL⁻¹)
249 for the control to 5.1% (g.100 mL⁻¹) for the PEF-assisted fermentation. Thus, for the PEF-
250 treated suspensions, the glucose consumption rates were significantly higher than those of the
251 control fermentation (Table 1). This could be explained by the greater yeast concentration,
252 which induces higher glucose consumption, especially during the Log phase where the cells
253 are exponentially duplicating. The results concur with those of McCabe, Barron, McHale, &
254 McHale, (1995), who reported 40% increase in the cellobiose conversion for *Kluveromyces*
255 *marxianus* cells under pulses of 0.25 kV for 10 ms. Also, the absorption of nutrients could be
256 enhanced after PEF-treatment by the improvement of the transport through the cell
257 membrane. This could be mainly attributed to the pore formation and/or the activation of
258 membrane proteins (Castro et al., 2005). The biomass yield on glucose was significantly

259 higher for the treated samples after 8 h of fermentation (Table 1), which confirms the above-
260 mentioned statements.

261 Aforementioned, the impact of PEF on the yeast size was studied. Figure 5 shows a
262 significant ($p < 0.05$) size increase of the cells after PEF treatment ($5.6 \pm 0.4 \mu\text{m}$), compared to
263 the untreated cells ($4.1 \pm 0.2 \mu\text{m}$). This observation concurs with the significant increase in the
264 biomass yield (Y ($\text{g}_{\text{biomass}} \cdot \text{g}^{-1}_{\text{glucose}}$)) where the mass of biomass per gram increases for one
265 gram of glucose consumed ($0.1 \text{ g}_{\text{biomass}} \cdot \text{g}^{-1}_{\text{glucose}}$) for the samples treated by PEF at $285 \text{ V} \cdot \text{cm}^{-1}$
266 compared to the untreated ones ($0.075 \text{ g}_{\text{biomass}} \cdot \text{g}^{-1}_{\text{glucose}}$) (Table 1). The different calculations
267 show an increase by 85% in the amount of biomass in gram compared to 75% increase in the
268 number of cells per mL, which indicates a 10% increase in the cell volume after PEF
269 treatment at $285 \text{ V} \cdot \text{cm}^{-1}$. It could be also noted that the treatment did not induce irreversible
270 deformations of the yeasts. The SEM photos show also that the cells kept their general shape
271 after the treatment. The review of the literature shows that smaller cell size was observed for
272 PEF-treated *S. cerevisiae* compared to the untreated ones (Araújo, Coelho, Margarit, Vaz-
273 junior, & Rocha-Leão, 2004). This observation was not observed for the strain
274 *Hanseniaspora* sp., studied here.

275 An important factor influencing the microorganisms' stimulation by PEF is the growth
276 phase. It has been previously demonstrated that PEF has different impacts on
277 microorganisms, depending on the growth phase (McDonald, Lloyd, Vitale, Petersson, &
278 Innings, 2000). In this line, the impact of PEF ($285 \text{ V} \cdot \text{cm}^{-1}$) on the growth and glucose
279 consumption kinetics was studied during each Lag and Log phases of the growth of
280 *Hanseniaspora* sp. yeast. When the suspensions were treated during the first 3 h (Lag and
281 early exponential phase), an increase in the yeast biomass concentration was observed. In
282 fact, after 8 h fermentation, the biomass concentration was increased by 60 % for the PEF-
283 treated suspensions during this phase (Figure 6). Lower yeast stimulation impact (25%) was

284 observed for the suspensions treated only during the Log phase. Besides, faster sugar
285 depletion was observed during the Lag phase, compared to the Log phase (Figure 6), with a
286 significant difference in the glucose consumption rates (Table 1). Even though, the sugar
287 consumption rates were significantly higher than the control for both PEF-treated
288 suspensions.

289 The results obtained show that *Hanseniaspora* sp. yeast cells were sensitive to the PEF
290 treatment during both Lag and Log phases, with more sensitivity during the Lag and early
291 exponential phase. These results concur with those reported by Mattar et al., (2013) who
292 observed a noticeable enhancement of the fermentation kinetics when stimulating *S.*
293 *cerevisiae* cells by PEF, with a significant acceleration of the sugar consumption during the
294 Lag phase. In addition, it was noted that the electroporation was more effective when the PEF
295 was applied during the first hours of fermentation rather than that after 24 h. It seems that the
296 cells became more resistant to electroporation for longer preliminary fermentation.

297 Regarding the energy consumption, it was obvious that the treatment at 285 V.cm^{-1} for 6
298 h consumed the highest energy, compared to the control and the other PEF-assisted
299 fermentations (Table 1). However, it provided the fastest cell growth and glucose
300 consumption kinetics. Overall, the results obtained in this work demonstrated the great
301 benefit of stimulating *Hanseniaspora* sp. yeast by PEF as it involves reducing the
302 fermentation time along with increasing the biomass concentration, and the low consumption
303 of energy.

304

305 **4. Conclusion**

306 In this work, the effects of PEF electrostimulation on *Hanseniaspora* sp. yeast cells were
307 studied. For an effective treatment time of 10.716 s, applied during both the Lag and Log

308 phases (6 h), the yeast concentration and the biomass yield increased significantly. The
309 depletion of nutrients was also significantly faster. The yeast cells were more sensitive to
310 PEF treatment during the Lag and early exponential phase rather than during the Log phase.
311 The yeast concentration and the biomass yield increased significantly during the Lag and
312 early exponential phase. However, the highest stimulation impact was observed using 285
313 V.cm⁻¹ electric field intensity applied during both phases. Increased growth rates were
314 accompanied by a faster depletion of glucose. The results obtained in the current study
315 indicate the great potential for the practical implementation of PEF-assisted fermentation
316 technology to improve yeast performances. However, further investigations are required to
317 understand the mechanisms behind microbial stimulation by PEF.

318

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324

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444 **Figure captions:**

445 **Figure 1. A.** Control batch mode fermentation without medium circulation, **B.** Control batch
446 mode fermentation with medium circulation (without PEF treatment), **C.** Schematic diagram
447 of the designed PEF-treatment chamber designed specifically for the current study, **D.** Real
448 photo of the PEF-treatment chamber.

449

450 **Figure 2. A.** A schematic representation of the PEF-assisted fermentation processes. **B.** PEF-
451 treatment parameters applied for *Hanseniaspora* sp. yeast stimulation.

452

453 **Figure 3. A.** Growth kinetics (viable cells) for the control and the PEF-assisted
454 fermentations. **B.** Optical densities at 600 nm for the control and the PEF-assisted
455 fermentation processes treated during the first 6 h and conducted until 8 h of fermentation.
456 Each fermentation experiment was repeated three times; the error bars correspond to the
457 standard deviations of each measurement.

458

459 **Figure 4. A.** Glucose consumption kinetics for the control and the PEF-assisted
460 fermentations. **B.** Soluble compounds depletion kinetics for the control and the PEF-assisted
461 fermentations. Each fermentation experiment was repeated three times; the error bars
462 correspond to the standard deviations of each measurement.

463

464 **Figure 5.** Scanning electron microscopy pictures of *Hanseniaspora* sp. yeast after 6 h
465 fermentation. **A.** Without PEF treatment, **B.** With PEF treatment at 285 V.cm⁻¹ field intensity.

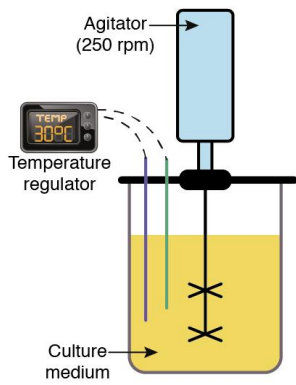
466

467 **Figure 6. A.** Growth kinetics of the control and the PEF-assisted fermentations at 285 V.cm^{-1}
468 field intensity, during the “Lag and early exponential phase” and “Log phase”, and during
469 both phases. **B.** Optical density measurements at 600 nm for the control and the PEF-assisted
470 fermentations (285 V.cm^{-1}). **C.** Glucose consumption kinetics for the control and the PEF-
471 assisted fermentations (285 V.cm^{-1}). **D.** Soluble compounds depletion kinetics for the control
472 and the PEF-assisted fermentations (285 V.cm^{-1}).

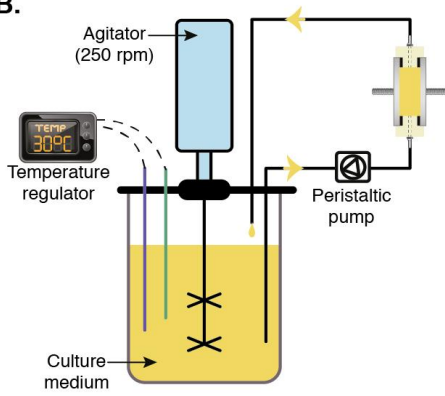
473 **Figures**

474 **Figure 1.**

A.

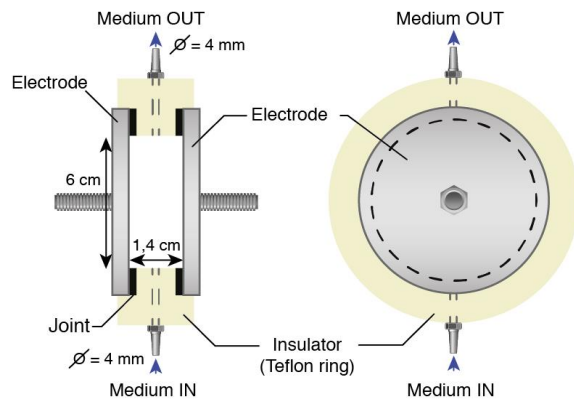


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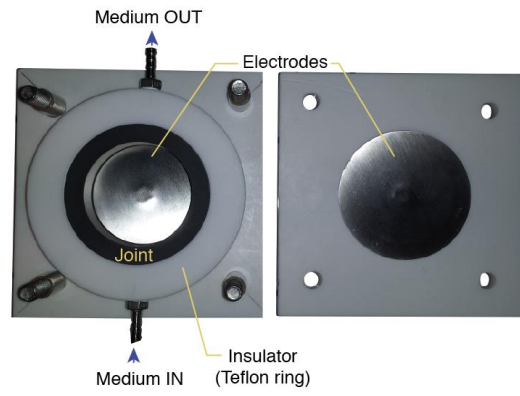


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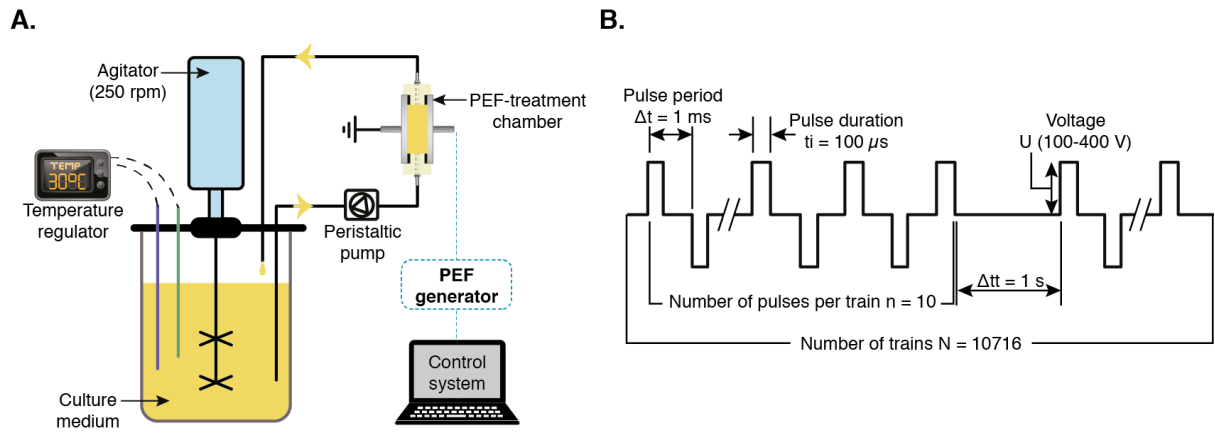
C.



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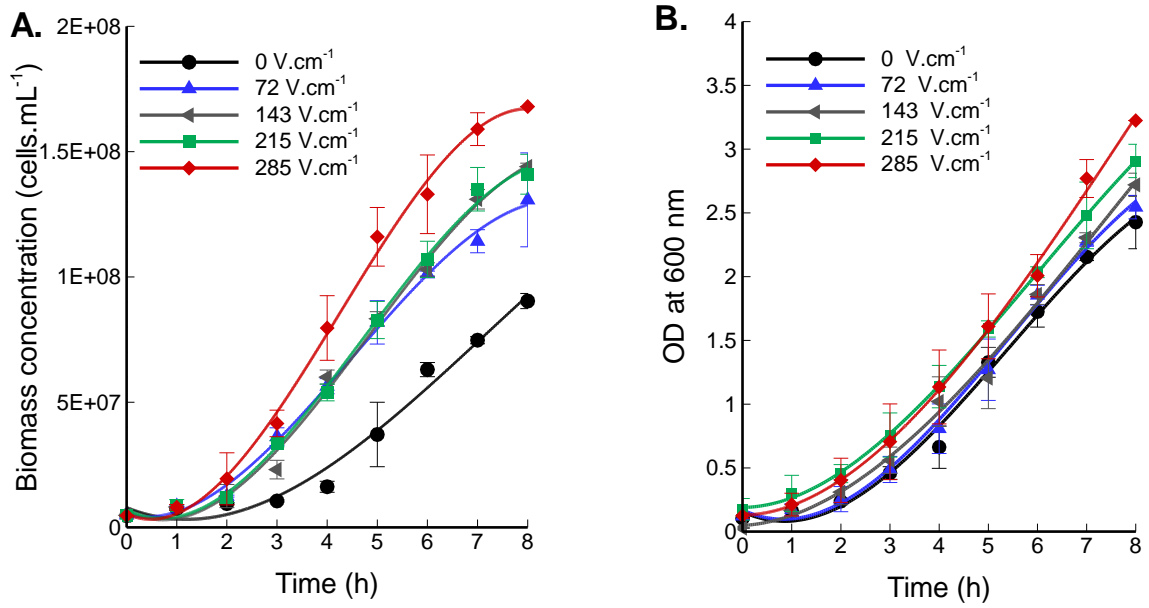


476 **Figure 2.**



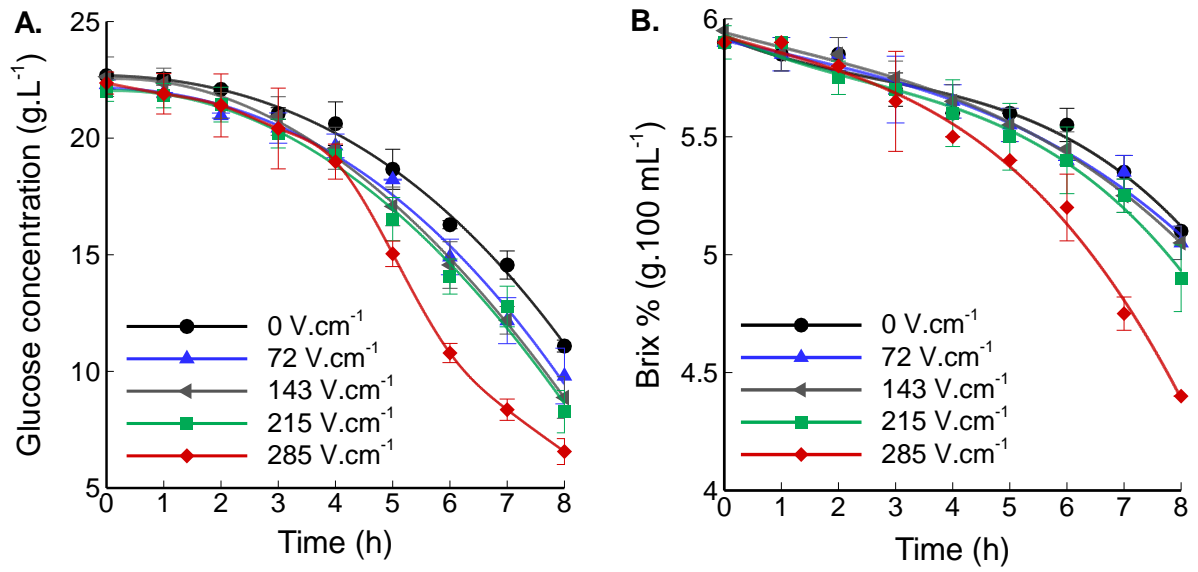
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478 **Figure 3**



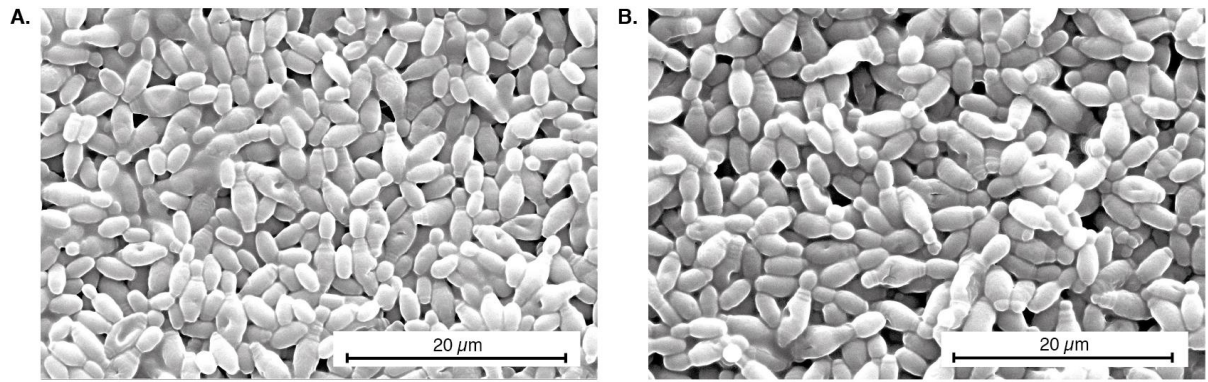
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480 **Figure 4**



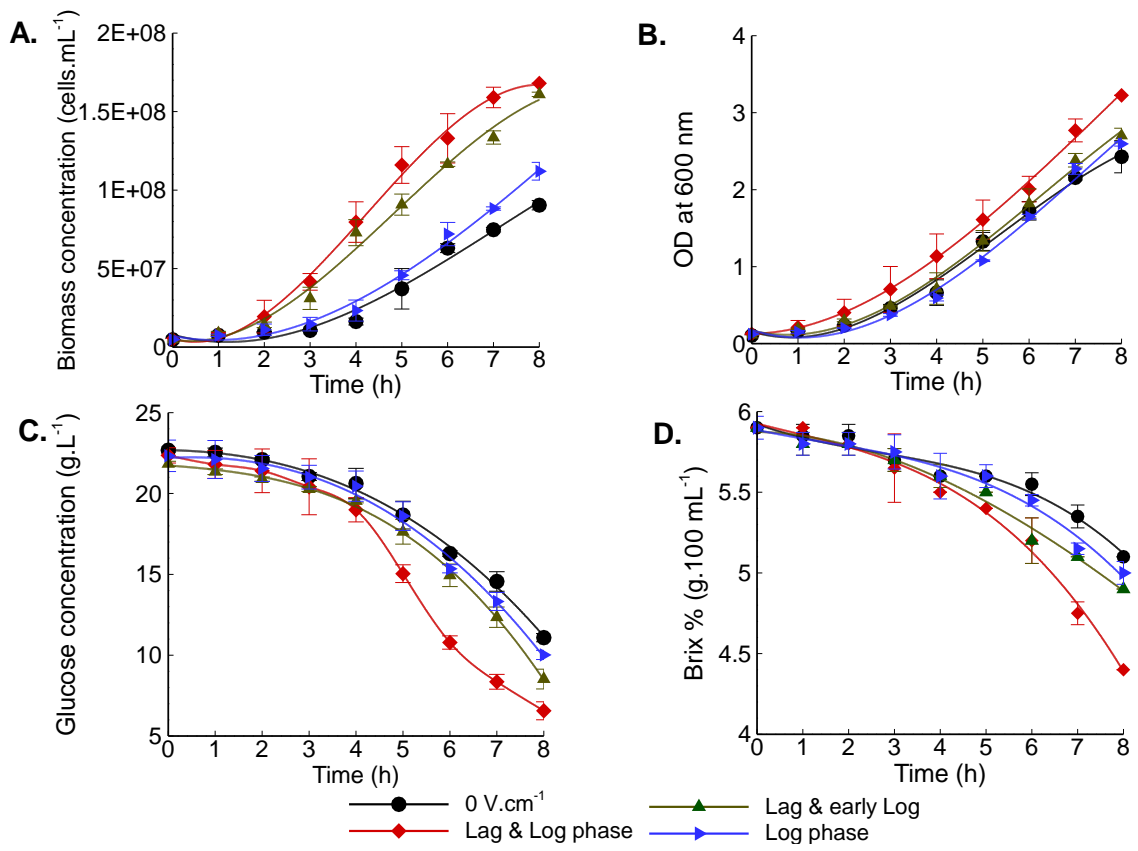
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482 **Figure 5**



483

484 **Figure 6.**



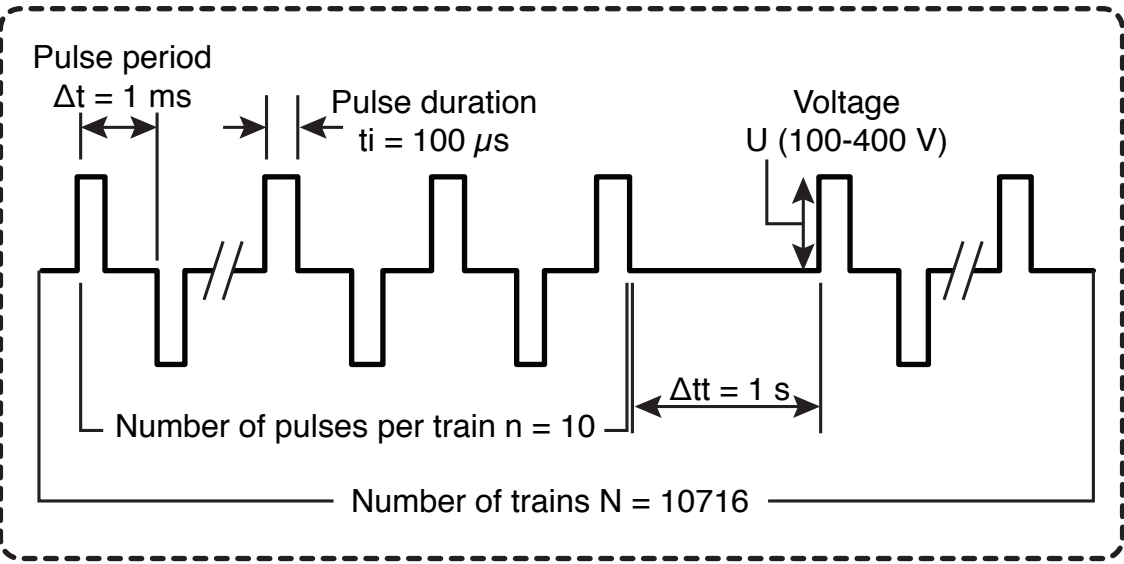
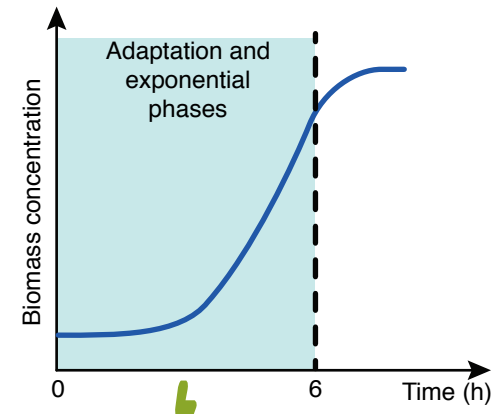
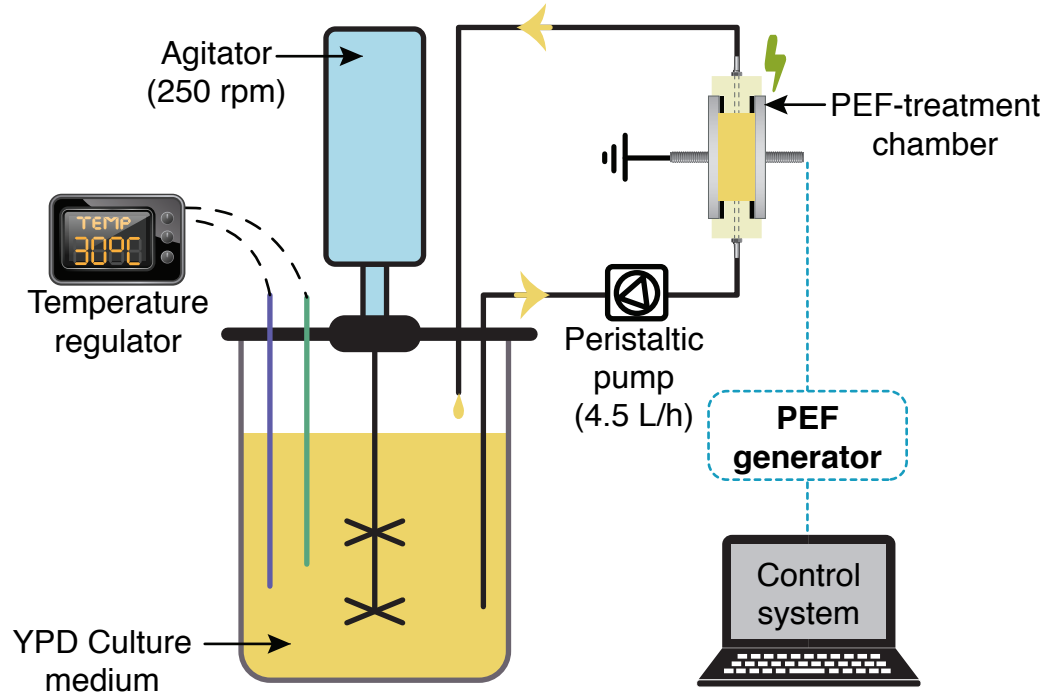
485

486 **Tables**487 **Table 1.** Energy consumptions, and fermentation process performances ($Y_{(g \text{ biomass/ g glucose})}$, dS/dt , μ_{max}) during PEF treatments.

	Lag and Log phases treatments					Lag and early exponential phase treated 285 V.cm ⁻¹	Log phase treated alone 285 V.cm ⁻¹
	0 V.cm ⁻¹	72 V.cm ⁻¹	143 V.cm ⁻¹	215 V.cm ⁻¹	285 V.cm ⁻¹		
W (J.mL ⁻¹)	N.A.	5.0 ± 0.4	22.0 ± 0.8	44.0 ± 1.03	82.0 ± 1.4	41.0 ± 0.9	42.0 ± 0.5
Y (g biomass/ g glucose)	0.075 ± 0.003	0.090* ± 0.002	0.090* ± 0.005	0.090* ± 0.003	0.100* ± 0.001	0.110* ± 0.001	0.090* ± 0.007
dS/dt (g.L ⁻¹ .h ⁻¹)	1.43 ± 0.02	1.56 ± 0.12	1.74 * ± 0.02	1.74 * ± 0.03	1.98 * ± 0.01	1.66 * ± 0.06	1.54 ± 0.08
μ_{max} (h ⁻¹)	0.39 ± 0.004	0.42 ± 0.01	0.43* ± 0.005	0.43* ± 0.008	0.47 * ± 0.01	0.45* ± 0.004	0.41 ± 0.003

488 The * denotes significant differences, N.A. denotes non-applicable

489



Optimal PEF intensity

