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1 **High-frequency, high-intensity electromagnetic field**
2 **effects on *Saccharomyces cerevisiae* conversion yields**
3 **and growth rates in a reverberant environment**

4 Emmanuel Bertrand^{a,b,d*}, Christophe Pasquier^{a,c}, David Duchez^{a,b}, Sebastien Girard^{a,c},
5 Agnès Pons^{a,b}, Pierre Bonnet^{a,c}, Catherine Creuly^{a,b} and Claude-Gilles Dussap^{a,b}

6 ^a Université Clermont Auvergne, CS 60032, 63001 Clermont-Ferrand, France

7 ^b Institut Pascal, UMR CNRS 6602 Team GePEB, Chemical Engineering, Applied
8 thermodynamics and Biosystems – BP 10448 F-63000 Clermont-Ferrand, France

9 ^c Institut Pascal, UMR CNRS 6602 Team PHOTON, Photonics, Waves, Nanomaterials –
10 BP 10448 F-63000 Clermont-Ferrand, France

11 ^d Aix-Marseille Université, INRA, Polytech' Marseille, UMR 1163 Biodiversité et
12 Biotechnologie Fongiques, 163 Avenue de Luminy, CP225, 13288 Marseille Cedex 09,
13 France

14

1 *Corresponding author: Tel: +33 (0) 491 828 568;

2 **Permanent address: emmanuel.bertrand@univ-amu.fr (E. Bertrand)**

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15 **Abstract**

16 Studies of the effects of electromagnetic waves on *Saccharomyces cerevisiae* emphasize
17 the need to develop instrumented experimental systems ensuring a characterization of
18 the exposition level to enable unambiguous assessment of their potential effects on liv-
19 ing organisms. A bioreactor constituted with two separate compartments has been de-
20 signed. The main element (75 % of total volume) supporting all measurement and con-
21 trol systems (temperature, pH, agitation, and aeration) is placed outside the exposure
22 room whereas the secondary element is exposed to irradiation. Measurements of the me-
23 dium dielectric properties allow the determination of the electromagnetic field at any
24 point inside the irradiated part of the reactor and are consistent with numerical simula-
25 tions. In these conditions, the growth rate of *Saccharomyces cerevisiae* and the ethanol
26 yield in aerobic conditions are not significantly modified when submitted to an electro-
27 magnetic field of 900 and 2400 MHz with an average exposition of 6.11 V.m⁻¹ and 3.44
28 V.m⁻¹ respectively.

29 **Highlights:**

- 30 • Rational method to assess the intensity and specific absorption rate in submerged
31 cultures developed
- 32 • The average mean electric field in submerged culture is respectively 6.11 and
33 3.44 V.m⁻¹ and the maximum local SAR is respectively 0.040 and 0.080 W.kg⁻¹ at
34 900 and 2400 MHz
- 35 • Liquid culture medium dielectric properties significantly limit the yeast exposi-
36 tion to the electromagnetic field
- 37 • No effect on growth rate and ethanol yield noticed

38 • Possible effect on glycerol and acetate yields to be further investigated

39 **Keywords:** Metabolic sensitivity to electromagnetic field; dielectric properties;

40 *Saccharomyces cerevisiae*; ethanol production; mode-stirred reverberation chamber

41 (MSRC).

42 **1. Introduction**

43 Electromagnetic stimulations are potentially effective to elicit either positive or adverse
44 effects in many biological systems (Pilla and Markov, 1994). Given the diversity of
45 exposition devices, frequencies, intensities, and durations used, the observed results
46 appear sometimes contradictory (Hristov and Perez, 2011). In modern societies, the
47 negative influence of intensive electromagnetic environment is one of the main
48 concerns of the citizens in terms of public health. The French National Agency for
49 Food, Environmental and Occupational Health and Safety (ANSES) stresses the need
50 for better characterized experimental devices to ensure the accurate determination of the
51 level of exposure to the electromagnetic fields to ensure an unequivocal assessment of
52 their potential effects on living cells (ANSES, 2013). In any case, there is a considerable
53 interest to improve a thorough understanding of the effects of electromagnetic radiation
54 on living cells.

55 It is noteworthy that biofuels are a credible alternative for a more sustainable future with
56 an important growth potential: in 2012 more than 93% of the 2500 millions of tons of
57 oil equivalent used for transportation came from non-renewable feedstock (IEA, 2012)
58 letting plenty of scope for improvements. It is also admitted that biomass production
59 processes are requiring major improvements to make them economically competitive
60 with their fossil equivalents (Menten, 2013; Chovau et al., 2013). Whatever the
61 generation of bioethanol, *Saccharomyces cerevisiae* is the microorganism of choice to
62 carry out alcoholic fermentation of sugars and has received considerable attention
63 (Bertrand et al., 2016). In terms of process intensification, two complementary
64 approaches are traditionally applied (Reay, 2008). Metabolic engineering and molecular
65 biology, aims at amplifying the metabolic fluxes leading to an overexpression of the

66 interesting metabolites or at gaining functionalities such as the consumption of a new
67 carbon source, for instance xylose to produce ethanol (Kim et al., 2013; Van Vleet and
68 Jeffries, 2009), while process engineering aims at preventing mass transfer limitations
69 (Hristov, 2010). Hunt et al. (2009) provide an extensive review of the use of
70 electromagnetic fields for biofuels and bioenergy applications and the underlying
71 possible mechanisms. In this context, the effects of electromagnetic fields in the ultra-
72 high-frequency range (within the 300 MHz to 3 GHz bandwidth) have been few studied
73 and quantified, although permanent magnets (da Motta et al., 2008; Muniz et al., 2007),
74 predominant electric field at low frequencies (Perez et al., 2007), pulsed electric fields
75 (Mattar et al., 2015) and sonication (Sulaiman et al., 2011; Indra Neel et al., 2012) have
76 proven their potential for improving metabolites production. The mechanisms are not
77 fully understood but some general trends are underlined depending on the
78 electromagnetic frequency applied. Permanent magnets (i.e. static magnetic fields) have
79 a major effect on the phospholipids orientation in the cell membrane affecting its
80 biomechanical properties (Wang et al., 2014). They also help to preserve enzymes
81 conformation and counterbalance the negative side effect of high ethanol concentration
82 that alters cellular membranes and unfolds cytosolic globular enzymes (Lopes and Sola-
83 Penna, 2001). Electric fields at low frequencies enable ionic redistribution as well as a
84 higher potential difference in the cellular suspension. The effects of pulsed electric
85 fields stimulations are largely unknown. It is hypothesized that a specific yeast
86 subpopulation is favored knowing that cytoplasmic membrane modifications are
87 induced by temporary electroporation (Mattar et al, 2015). External electric fields
88 improve nutrient transportation through the modified cell membrane via pore formation
89 or even transport proteins activations (Castro et al., 2012). An improved gas-liquid mass

90 transfer for dissolved carbon dioxide removal, which is known to inhibit
91 *Saccharomyces cerevisiae* growth, is the main effect of Mild sonication on the culture
92 broth leading to higher ethanol productivity (Indra Neel et al., 2012).

93 Therefore, depending on their settings, electromagnetic stimulations have potential to
94 promote one or the other approaches for process intensification of *Saccharomyces*
95 *cerevisiae* fermentation. In the high-frequency range, previous studies showed that high-
96 frequency electromagnetic waves promote and stabilize free radicals in yeasts
97 associated with oxidative stress. This effect is amplified when the intensity of the field
98 increases (Crouzier et al., 2009). Oxidative stress can alter significantly the synthesis of
99 reduced metabolites via redox mechanisms. The regulation of NADH homeostasis
100 directly impacts redox balance and the metabolic fluxes distribution. According to Hou
101 et al. (2010), an increase in mitochondrial NADH promotes ethanol production by
102 restricting the entry of carbon into the TCA cycle while an increase in cytosolic NADH
103 leads to glycerol production. However, these findings have not yet been investigated for
104 bioethanol production improvements.

105 The two aims of this study are: (i) to develop and characterize a fully controlled and
106 instrumented bioreactor for the culture of cells in a liquid medium under controlled
107 electromagnetic stimulations; enabling both an off-line determination of metabolites
108 concentrations and online assessment of growth rates by gas-phase analysis and mass
109 balances. (ii) to master an intense oxidative stress on *Saccharomyces cerevisiae* cultures
110 using high-frequency, high-intensity electromagnetic field to potentially trigger an
111 overproduction of ethanol.

112 **1. Materials and Methods**

113 **1.1. Exposition to the electromagnetic field**

114 1.1.1. Dielectric measurements

115 For assessing the influence of electromagnetic stimulations one key variable is the
116 electric properties of the culture medium. This is obtained measuring the complex
117 permittivity (complex number).

118 The culture medium relative permittivity (ϵ_r) and electric conductivity (σ [$S.m^{-1}$]) were
119 measured using a “Di-line” Transverse Electromagnetic sensor (INDEXSAR,
120 Newdigate, United-Kingdom) at 30°C, the temperature set-point used for the culture. A
121 vector network analyzer (Rohde & Schwarz ZVB8) was used to measure the
122 transmission loss and phase of the TEM-line with or without the culture medium
123 making it possible to compute the dielectric properties for all the frequencies used.
124 Detailed explanations about the computation can be found in (Toropainen et al., 2000).

125 1.1.2. Mode Stirred Reverberation Chamber (MSRC)

126 To obtain a defined and controlled electromagnetic field, irradiated cultures have been
127 performed in a specific chamber. The MSRC is a special device to obtain reproducible
128 electromagnetic field generation (Fig. 1). A double-layered steel Faraday cage protects
129 the experiment from external or environmental electromagnetic field. An antenna (ETS
130 Lindgren 3144) is used to generate the electromagnetic field, while the walls and a
131 stirrer reverberated randomly the incident waves within the cavity. Within a specific
132 volume of the chamber, called working area (delimited by the blue points in Fig. 1), the
133 electromagnetic field is statistically (i.e. for a full rotation of the stirrer) homogeneous
134 and isotropic (Hill, 1994). The method is fully normative (IEC, 61000-4-21) and more
135 explanations concerning the Institut Pascal’s MSRC properties and performances for the
136 exposition of biological samples are available in (Lalléchère et al., 2010). The intensity
137 of the electromagnetic field impacting the bioreactor is measured using an isotropic
138 electric field antenna (PMM-EP 183).

139 1.1.3. Numerical Simulations

140 The main issue is to determine the accurate exposure field of the biomass submerged in
141 the culture medium. A commercial software based on the method of moments (FEKO
142 EM Software version 5.5, FEKO EM Software & Systems GmbH, Böblingen,
143 Germany) coupled with an analytic approach was used to estimate the electromagnetic
144 field impacting the culture compartment located inside the MSRC. For all the
145 simulations performed with FEKO, the unstructured mesh has been sized so that the
146 characteristic length is at least smaller than $\lambda/4$ at a frequency of 2400 MHz (the highest
147 frequency used in this study is fixing the finer mesh that is necessary). However, due to
148 calculation capacity limitations, numerical simulations of the reactor inside the MSRC
149 at scale 1 were not possible. To overcome this limitation, we used MATLAB R2017a
150 and obtained, from the theory of electromagnetic waves propagating inside a cavity the
151 analytical expressions of the electromagnetic field at any point inside the MSRC, for
152 each resonance mode of the chamber and for each propagation direction. The vectorial
153 sum of these fields was then calculated to obtain the distribution of the total field in the
154 MSRC for each Mode. As an example, Figure 2A represents the electric Field
155 repartition for mode 7/33/14 at 900 MHz. As the MSRC has losses, there is no Dirac
156 signal associated for a given Mode. It is observed a reduction in the signal amplitude
157 spreading around the reference frequency that can be easily approximated by a
158 Lorentzian (Fig. 2B) with the quality factor of the MSRC that has been previously
159 determined for each frequency. At 900 MHz (Fig. 2C) and at 2400 MHz (Fig. 2D),
160 several resonance modes are present in the MSRC. To accurately characterize the
161 electromagnetic field, it is necessary to consider the contribution of each of these
162 different Modes, which were also excited by the source at 900 or 2400 MHz. However,
163 their impact must also be weighed against their frequency distance from the reference

164 frequency. The number of modes has been limited to those that are less than 5 times out
165 of the Lorentzian bandwidth that is centered on the emission frequency of the source.
166 The circles indicate the weighting coefficient used for the field strength of each mode
167 considered. Once these modes have been determined, it is then possible to calculate the
168 total Electric Field resulting from the vector sum of all these contributions and to obtain
169 an evolutionary image of the amplitude of the E-field at the reactor site in the MSCR at
170 900 MHz (Fig 2E) and 2400 MHz (Fig 2F). The total computed electric field is
171 compared with the well-known MSCR characteristics at 900 MHz and 2400 MHz to
172 recalibrate the simulation results with the experimental conditions.

173 1.2. Cultures

174 *Saccharomyces cerevisiae* ATCC 7754 was kept at 4°C on an agar plate containing 3g
175 yeast extract, 3g malt extract, 5g peptone and 10 g glucose per liter. A single colony is
176 sub-cultured on a fresh plate and incubated for 48h at 30°C.

177 Culture media, 1 M NaOH solution (used for pH adjustment during the culture) and
178 Antifoam A (Sigma-Aldrich, Illkirch, France) were sterilized for 20 min at 121°C. The
179 inoculum was prepared by aseptically transplanting the yeast into a 500 mL Erlenmeyer
180 flask containing 200 mL of the culture medium. It was formulated according to
181 Kristiansen (1994) prescriptions to bring the strict necessary elements for the yeast's
182 growth (Glucose: 50 g·L⁻¹; KH₂PO₄ : 3g·L⁻¹, (NH₄)₂SO₄ : 2 g·L⁻¹; vitamins, salts and
183 minerals). The suspensions are then placed overnight at 30 °C under continuous shaking
184 (200 rpm). Glucose concentrations were set to 10 and 50 g·L⁻¹ for the inoculum and
185 bioreactor cultures respectively.

186 The culture was operated on a 500 mL working volume instrumented Multifors
187 bioreactor (Infors HT, Bottmingen, Swiss). The reactor is equipped with a pH probe, a

188 temperature controller, a sensor detecting the level of foam and a sensor measuring the
189 partial pressure of dissolved oxygen (pO₂). The process was regulated at 30°C, 600 rpm
190 and pH 5.5. The bioreactor was connected to a secondary reactor (placed in the MSRC
191 for the electromagnetic field stimulations) consisting in a glass column (internal
192 diameter 5 cm, length 25 cm for a total volume of 490 mL) through 2* 10 m of flexible
193 tubes (internal diameter 0.8 mm). A Masterflex (ref 7518-10) peristaltic pump (Cole
194 Palmer, Vernon Hills, IL) let the medium circulates within the whole system at a flow
195 rate of 288 mL·min⁻¹. A Luxtron SFT infrared pyrometer (LumaSense Technologies,
196 Santa Clara, CA) was used to assess the temperature of the secondary reactor during the
197 whole culture. Samples were taken out periodically and biochemical analyses were
198 carried out in duplicates.

199 1.3. Liquid phase analyses

200 Samples from the reactor were immediately filtered (on 0.2 µm hydrophilic filter) to
201 separate the medium from the biomass.

202 The dry cell weight was determined by measuring the optical density at 600 nm of a
203 yeast suspension with a spectrophotometer (BIOMATE 3S, ThermoFischer Scientific,
204 Illkirch, France) and correlated with 5 mL filtered and dried biomass (24 hours at 105
205 °C) weightings.

206 The filtrate was then deproteinized by the addition of 125 µL of barium hydroxide and
207 125 µL of zinc sulfate (5% w/v) and centrifuged at 10 000g for 5 minutes. The
208 supernatant was kept for the following analyses.

209 Organic acids, alcohols, and glucose were quantitated by HPLC. The separation is
210 carried out at 50°C in isocratic mode at an eluting flow rate of 0.7 mL·min⁻¹ of a 5 mM

211 sulfuric acid solution on two Phenomenex Rezex ROA (7.8 × 300 mm) columns
212 connected in series (Phenomenex, Le Pecq, France). Detection is performed by
213 refractometry while the acquisition and the integration of chromatographic peaks rely
214 on the HPCChem (ver. A.08.03) software (Agilent, Massy, France). Quantitation is
215 performed against a standard for the different compounds of interest. The detection
216 limits of the various products are between 1 mg and 0.1 g·L⁻¹.

217 Amino acids and ammonium ions were spectrophotometrically assayed according to a
218 modified ninhydrin (Moore & Stein, 1948) or phenol-hypochlorite (Weatherburn, 1967)
219 method respectively. Reactants were obtained from Sigma-Aldrich (St. Louis, MO,
220 USA) and were of analytical grade (purity > 99.5%).

221 The exponential growth rates are determined by regression analysis (linear estimation)
222 of biomass dry mass versus time in log scale. The production yields are determined by
223 plotting the total quantity of products formed as functions of the total quantity of
224 substrate consumed. The yields are determined by linear regression. The Monod
225 hypothesis, which assumes constant yields, is verified in the case of the substrate
226 concentration being tested. Standard deviations, variances and confidence intervals of
227 the identified parameters (maximum growth rates and reaction yields) were calculated
228 considering the most acceptable criterion is the mean square estimate.

229 In order to ensure the consistency of the various analyses carried out during the culture,
230 the conservation balances of carbon (B_C) and nitrogen (B_N) were calculated assuming the
231 following C molar formula for *Saccharomyces cerevisiae*: CH_{1.6}O_{0.52}N_{0.15}.

232 1.4. Gas phase analysis

233 The gas fraction was preliminarily dried in a drierite gas-drying unit (W. A. Hammond
234 Drierite Co, Xenia, Ohio) and the molar fraction of oxygen and carbon dioxide were
235 measured at the exhaust of the reactor using a ZRE gas analyzer (Fuji Electric,
236 Clermont-Ferrand, France). Gaseous balances on the reactor lead to the instantaneous
237 respiration rates according to the following equations:

238 G^e is the air molar flow rate feeding the reactor expressed in $\text{mmole}\cdot\text{h}^{-1}$. V_r is the
239 volume of the culture medium in the reactor at a specific time t . It is the initial volume
240 of culture medium corrected with the volume of the different samples taken out during
241 the culture and with the volume of the sodium hydroxide solution that has been added to
242 the vessel to keep the desired pH. r_{O_2} and r_{CO_2} are the specific oxygen consumption and carbon
243 dioxide production rates expressed in $\text{mmol}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$. $y_{O_2}^e$ is the molar oxygen fraction in the
244 air feed (≈ 0.2093), while $y_{O_2}^r$ and $y_{CO_2}^r$ are the molar fraction of oxygen and carbon dioxide in
245 the dried air leaving the reactor.

246 The amounts of consumed oxygen and produced carbon dioxide expressed in mmol L^{-1}
247 during the culture are calculated by integrating the instantaneous respiration rates
248 according to the following equations:

249 For convenience, this integration was carried out digitally after discretization of a time
250 interval $\Delta t = 20\text{s}$ corresponding to the acquisition rate being set for the gas analyzer. The
251 respiratory quotient RQ can then ultimately be calculated:

252 2. Results and Discussion

253 2.1. Exposition to the electromagnetic radiations

254 One of the fundamental issues is the selection of the most suitable exposure facility
255 (Paffi et al., 2010). The actual electromagnetic environment is far more complex than a
256 planar irradiation that is conventionally used for most of the bio-electromagnetism
257 studies. In this context, the Mode Stirred reverberation chamber (MSRC) has proved to
258 be an efficient tool for bioelectromagnetism stimulations (Lalléchère et al., 2010).
259 Previous experiments carried out on vegetables such as *Lycopersicon esculentum* have
260 proved the potential to induce transcription, translation and even ATP concentration
261 changes at high-frequency and even low intensity such as $5 \text{ V}\cdot\text{m}^{-1}$ (Roux et al., 2007).
262 However, this type of environment cannot accommodate with any type of electrical
263 devices whose presence is likely to alter the ideal distribution of the electromagnetic
264 field. Conversely, electronic cards may be damaged by an exposition to intense
265 electromagnetic radiations. A reproducible microbial culture must necessarily be carried
266 out in a regulated and controlled bioreactor. However, the presence of the entire system
267 including all the regulation devices inside the exposure chamber was not an option as
268 the electromagnetic field might damage the electronic parts of the system. Therefore,
269 the instrumented bioreactor was coupled with a secondary exposition vessel to examine
270 the biological effects of the electromagnetic field. The suspension was recycled through
271 the MSRC as shown in Fig. 3. The connecting pump flow rate is $288 \text{ mL}\cdot\text{min}^{-1}$. Under
272 these conditions, the residence time in the secondary element is 94s (0.45 L), which
273 corresponds to an exposition to the electromagnetic waves for 25% of the culture time.
274 In the other parts (including the main culture vessel and the connecting parts) the
275 residence time was 280s (1.35 L).

276 2.2. Measurements of the medium dielectric properties

277 A frequency scanning from 600 to 2500 MHz (Fig. 3) indicates that the relative
278 permittivity of the yeasts suspension (minimal medium) is slowly decreasing from 78 to
279 74 while the electric conductivity is sharply increasing from 0.4 to 2.5 S·m⁻¹. These
280 measurements agree with the behavior of an electrolyte solution such as saline water
281 (Gadani et al., 2012). Additionally, to the frequency being applied, the initial
282 composition of the culture medium is of importance. For instance, the addition of
283 ammonium sulfate into the minimal medium, which aims at bringing nitrogen into
284 available form, also brings sulfate as a counter ion. This has the side effect to
285 considerably increase the medium conductivity. The measurements have shown that at
286 any frequency the conductivity for the minimal medium is about 0.5 S·m⁻¹ higher than
287 the one for the yeast medium. The production of biomass and metabolites (ethanol,
288 glycerol, acetate) during the culture are also susceptible to continuously modify the
289 dielectric properties of the culture medium. At lower frequencies, in the kHz range near
290 the β -relaxation zone, these properties are exploited to develop online biomass sensors
291 (Martisen, Grimmes and Schwan, 2002). However, a preliminary study has shown that
292 these changes in the high-frequency range (600 to 2500 MHz) have no significant effect
293 on the medium conductivity but may lead to a 10% increase of the medium permittivity.
294 The filtration of the medium for removing the biomass also indicates that these changes
295 are related to the metabolites and not to the presence of the yeasts themselves in the
296 medium (data not shown). Starting the fermentation with a 50 g·L⁻¹ glucose
297 concentration does not lead to any significant changes in the medium dielectric
298 properties (Fig 3). Therefore, the exposition to the electromagnetic field could be
299 considered identic throughout the culture. It is further noteworthy to mention that a
300 higher conductivity leads to a higher attenuation of the electromagnetic waves inside the

301 medium. This had an impact on the reactor design. Its shape must be thin enough to
302 permit a homogenous exposition of the yeasts to the electromagnetic field.

303 2.3. Numerical Simulations in an anechoic environment
304 Numerical simulations were used to assess the influence of the geometry, the material
305 properties and of the thickness of the secondary reactor element and their impact on the
306 electromagnetic waves propagation. Due to the complexity of the real electromagnetic
307 environment, a straightforward approach was to consider the incident electromagnetic
308 field as a plane wave. The assumption is thus to consider the culture is in an anechoic
309 environment. Using FEKO electromagnetic simulation software, various reactors
310 configurations were illuminated by a plane wave at the two frequencies selected for the
311 study: 900 MHz and 2400 MHz. Results from these preliminary simulations indicate
312 that the use of Pyrex glass or polyethylene does not induce any significant changes in
313 the electromagnetic profile observed in the culture medium. The selected materials
314 appeared globally transparent in the frequency range studied.

315 The simulation of electromagnetic field propagation at 900 MHz and 2400 MHz has
316 different profiles for the same incident power. The maximum amplitude of the electric
317 field penetrating inside the reactor is 3 times more intense in the case of the exposition
318 at 900 MHz than with the exposition at 2400 MHz. This is both due to the electric
319 conductivity increase in the medium, from 0.9 to 2.3 S.m⁻¹ (Fig 3.) and the frequency
320 shift (skin effect). It can be noticed that the intensity of the field is truly heterogeneous
321 in the anechoic configuration as it can be seen in the cross sections presented in figure
322 4A.

323 In order to validate these simulations results, a direct measurement of the
324 electromagnetic field in the culture medium has been performed. The experimental

325 measurement of an electromagnetic field inside a liquid is in itself a challenge. In fact,
326 as small as the probes may be, these latter are intrusive and not pointwise. Therefore,
327 the measurement is the result of integration over a finite volume element. It is therefore
328 not possible to access the measurement with a millimetric (or even centimetric) spatial
329 resolution. This is even accentuated when the field intensity rapidly changes inside the
330 measurement volume. Moreover, the probes are generally developed in order to perform
331 field measurements in the air and not directly into liquids. Before being immersed in
332 culture broth, the probe should be protected in a sealed glass tube, which then creates
333 new interfaces and further possible electromagnetic reflections. Finally, the probe itself
334 can interfere with the electromagnetic environment. In this case, it is likely that the
335 electric field intensity measured by the probe is not that prevailing in its absence. Thus,
336 the effect of the perturbation introduced by the probe on the electromagnetic
337 environment must be investigated numerically.

338 Experimental measurements were performed in the empty reactor and after having it
339 filled with the medium so that the position of the probe has not been modified. The
340 results indicate that 68 % of the electric field amplitude measured in the empty reactor
341 is effectively transmitted at the center of the culture medium. These experimental results
342 are not in agreement with the numerical simulations that indicate that 18% of the
343 incident field is effectively transmitted (Fig. 4B). This discrepancy has been explained
344 by the impact of the insertion of the probe into the reactor, proving an experimental bias
345 introduced by the probe. Four simulations have been performed successively. The first
346 one concerns the empty reactor. The second simulation accounts for the effect of the
347 liquid medium electromagnetic properties when the reactor is filled with the culture
348 medium. The third simulation introduces a fictive glass cylinder filled with air,

349 simulating the tube protecting the measuring probe. The fourth simulation accounts for
350 a small diameter conductive cylinder in to simulate the probe itself (Fig 4B). The
351 simulation results show that the effect of the probe agrees with the measured values.
352 This permits to validate the simulation results and confirm the calculation of the electric
353 field that is transmitted to the cells in culture conditions, i.e. 18 %, whereas the
354 experimental value is 68 % due to probe effects.

355 The simulation of the impact of the probe tends to strengthen the confidence in the field
356 values determined numerically and indicates that the field experimental values obtained
357 by the probe are not fully representative of the level that is actually perceived by the
358 culture medium in the irradiated part due to the modification of the electromagnetic
359 environment introduced by the probe itself. The recent commercialization of sub-
360 millimeter electro-optical sensor, adapted to the field of measurement in liquid media
361 and even plasma might be a valuable solution to improve the experimental mapping of
362 field profiles in the irradiated part of the reactor (Jarrige et al., 2012).

363 2.4. Electromagnetic field propagation inside the yeast itself

364 Depending on the frequency, the propagation of the electromagnetic field inside the
365 cells might schematically vary between two extreme schemes. Either the wave passes
366 through the yeast cells without any attenuation, or for the lowest frequencies, the yeast
367 will be shielded and therefore preventing any possible action of the electromagnetic
368 field (Hristov and Perez, 2011). Advances in dielectrophoresis (Asami, 2002) associated
369 with single cell impedance spectroscopy make it possible to extract with a relatively
370 good accuracy both relative permittivity and medium electric conductivity at subcellular
371 lever for frequencies from 0 up to 500 MHz (Haandbæk et al., 2014). Given technical
372 limitations, there does not exist any data at subcellular scale for frequencies higher than

373 500 MHz. Therefore, these values must be considered the best estimations currently
374 available. Based on these data, the propagation of the electromagnetic field waves has
375 been simulated inside semi-infinite planes having the dielectric properties of the diverse
376 cellular sub-compartments. The results indicate that the electromagnetic wave
377 propagates very efficiently inside the cell wall, is only slightly attenuated by the cell
378 membrane and is mostly attenuated by the cytoplasm of the cell. Given the size of the
379 yeast, we have estimated that more than 99% of the electromagnetic field impacting the
380 yeast passes effectively through. Another important fact is that the dielectric properties
381 of the cytosolic compartment determined by Haandbæk et al. study, (2014) are close to
382 that observed for the culture medium. Hence, it seems appropriate to approximate the
383 specific absorption rate of a yeast suspension by that of the culture medium in the
384 irradiated part of the reactor.

385 2.5. Numerical study in the reverberant environment

386 Since the reactor temperature is regulated, it is not possible to evaluate the specific
387 absorption rate (SAR) by measuring the temperature rise of the culture medium.
388 Besides, infrared monitoring of the temperature in the irradiated part of the reacting
389 volume showed no significant variations during the illumination (compared to the
390 control culture). To estimate the SAR and its maximum value, the electromagnetic field
391 strength in the reverberant environment must be calculated according to the dielectric
392 properties of the culture medium. For computation capabilities reasons it was not
393 possible to simulate the Institute Pascal experimental MSRC (L = 8.4 m; l=6.7 m, H =
394 3.5 m) directly at the chosen frequencies. In order to determine the field cartography
395 and to estimate the SAR, FEKO simulations in free space and an analytic approach were
396 combined. First, using FEKO the reactor was illuminated with three different waves at

397 900 MHz and 2400 MHz in free space. These directions represent the three directions of
398 the different modes present in the MSCR. The profiles of the simulated electric field at
399 900 MHz and 2400 MHz according to these three directions are presented in Fig. 5.
400 From these results, it was possible to determine the maximum percentage of the electric
401 field penetrating the reactor. According to the simulations, the maximum value of 95 %
402 was obtained. This value has been taken as a reference to compute the electric field
403 intensity inside the MSCR according to the analytic approach presented previously.
404 Secondary knowing the percentage maximizing the effects inside the reactor, it was
405 possible to calculate the local SAR (SAR without mass or volume) inside the reactor for
406 20 000 points chosen arbitrarily in the reactor volume. SAR and local SAR expression
407 are given by the following equations:

408

409 The repartition of the different values of the 20 000 random positions in the reactor is
410 given in Fig. 5E for 900 MHz and Fig. 5F for 2400 MHz. The mean field strength, the
411 average local SAR and the maximum local SAR corresponding to the experimental
412 conditions are given in Table 1.

413 Values obtained are far below the threshold of 2 W.kg^{-1} that is normally used to
414 distinguish non-thermal and thermal effects. It should also be noted that the yeast cells
415 are also set into motion by the hydrodynamics of the system, which is also contributing
416 to the exposure homogeneity although it was not possible to model this numerically.

417 2.6. *Saccharomyces cerevisiae* batch culture results

418 The control experiment was carried out by maintaining the recycling loop during the
419 culture without any application of the electromagnetic field inside the MSRC. The same

420 procedure has been applied for irradiated experiments except for the switching-on of the
421 device for generating electromagnetic waves. The results are reported and compared in
422 table 1 and Figure 6. The yields and the maximum growth rate are calculated by linear
423 regressions. The growth rates and ethanol yields reported in this study are typical for
424 Crabtree positive yeasts operated under aerobic conditions. It leads simultaneously to
425 high fermentation yields (0.4 g per g of substrate) and relatively low biomass yields
426 (0.13g per g of substrate) as reported by Albergaria and Arneborg (2016).

427 Contrary to the results observed with permanent magnets or at low frequencies magnetic
428 waves by Da Motta et al. or Perez et al. (2007), there is no evidence for an effect both
429 on the maximum growth rate and on the main conversion yields between the main
430 components (biomass, glucose, ethanol, O₂ and CO₂), considering the standard
431 deviations of the identified parameters. This means that the specific rates remain
432 identical in exposed and in reference non-illuminated conditions. Contrarily, the
433 glycerol and acetate yields seem very slightly affected by irradiation, by a factor of 1.2-
434 1.5. This may be related to a perturbed status of redox intracellular balance due to the
435 electromagnetic field. However, the global carbon and nitrogen balances do not appear
436 to be affected, given the accuracy of the measurements. As the various reactions yields,
437 as well as the respiratory coefficient, do not indicate any significant change, it is
438 considered that the global stoichiometry of growth and of the production of primary
439 metabolites by *Saccharomyces cerevisiae* are not influenced by an exposure of 25% of
440 the culture vessel to high-frequency high-intensity electromagnetic fields. These results
441 are close to the reported results obtained by Anton-Leberre et al. (2010) who did not
442 find any effect of high static or even pulsed magnetic fields on the cellular processes of
443 the yeast *Saccharomyces cerevisiae*. Nevertheless, this study brings a rational estimation

444 of actual volume, intensity, specific absorption rate and distribution of electromagnetic
445 fields inside the culture medium, confirming the observation given by Anton-Leberre et
446 al. (2010) with strictly controlled and quantified electromagnetic field conditions.

447 2.7. Recommendation and perspectives for further studies for
448 assessing the effects of electromagnetic fields in submerged cultures
449 The study on the effect of electromagnetism waves impacting a living organism requires
450 a specific and adequately characterized experimental device. The development of such a
451 system has been done. Three important remarks must be done: (i) the exposure time is
452 limited in comparison with the total culture time (about 25% in the present study); (ii)
453 the intensity of the electromagnetic field actually transmitted to the cells is significantly
454 attenuated in comparison to that impacting the irradiated part of the reactor (30% at 900
455 MHz and less than 20% at 2400 MHz) in a anechoic environment; (iii) the direct
456 experimental measurement of the intensity of the electromagnetic field into the
457 fermenting medium is technically impossible due to the changes of the confined
458 electromagnetic environment caused by the probe itself. Direct measurements provide
459 over-estimated values of the electric field intensity, ignoring the shielding effect of the
460 aqueous medium itself. This requires the systematic use of numerical simulations for
461 assessing the actual field of radiation perceived by the cells. This also underlines the
462 need for sensors (such as electro-optic probes) capable to function in a liquid medium
463 without disrupting the overall electromagnetic environment. The use of an exposure
464 chamber in its reverberation configuration is mandatory since it achieves an
465 electromagnetic field strength that is substantially greater in the secondary reactor in
466 comparison from its anechoic configuration. Moreover, it allows obtaining a much more
467 uniform and homogeneous electromagnetic environment regarding the amplitude of the
468 field. Because of culture medium recirculation, the hydrodynamics inside the reactor as

469 well as the sinusoidal properties of the electric field, it must be considered that cells
470 have been statistically exposed in a much more homogeneous way, considering they are
471 crossing randomly the different regions of the liquid phase.

472 In the characterized electromagnetic environment as described previously, it has been
473 observed that no biological specific response on the biomass, glucose, ethanol, CO₂ and
474 O₂ yields was macroscopically observed at frequencies of 900 and 2400 MHz, with an
475 emitted intensity average intensity perceived by the cells of 6.1 V·m⁻¹ and 3.44 V·m⁻¹
476 respectively applied on 25 % of the reacting volume. This suggests that a high-
477 frequency, electromagnetic field does not induce any major modification of the ethanol
478 production rate by *Saccharomyces cerevisiae*. However, it is observed that the
479 conversion yields to glycerol and acetate decreased by a factor of 1.2 – 1.5 compared to
480 non-irradiated conditions. Further investigations would be needed to determine whether
481 the absence of observed biological effect is associated with a protecting role of the
482 liquid culture medium that behaves like a “high cut filter” thus significantly limiting the
483 yeast exposition to the electromagnetic field or to the absence of actual interaction
484 between the high-frequency electromagnetic fields with the metabolism of
485 *Saccharomyces cerevisiae*. In this context, the increase of the emitted power with the
486 use of a pulsed electromagnetic field, or the design of an exposure device associating an
487 internal source for the electromagnetic waves emission in a waveguide with specific
488 dimensions to select the pertinent wavelength could be an interesting option to
489 investigate.

490 **3. Conclusion**

491 The present study develops a rationale method for assessing rigorously the intensity and
492 the specific absorption rate (SAR) of electromagnetic waves in submerged cultures of

493 *Saccharomyces cerevisiae*. For the frequencies 900 MHz and 2400 MHz, the average
494 mean electric field was respectively 6.11 and 3.44 V.m⁻¹ and the maximum local SAR
495 was respectively 0.040 and 0.080 W.kg⁻¹ well below the onset of thermal effects. With
496 irradiation of 25 % of the culture medium, no effect on growth rate and ethanol yield is
497 noticed, though significant deviations are observed for glycerol and acetate yields,
498 showing a possible secondary effect on redox metabolic balance that needs to be further
499 investigated.

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502 "Investissements d'Avenir" through the IMobS3 Laboratory of Excellence (ANR-10-
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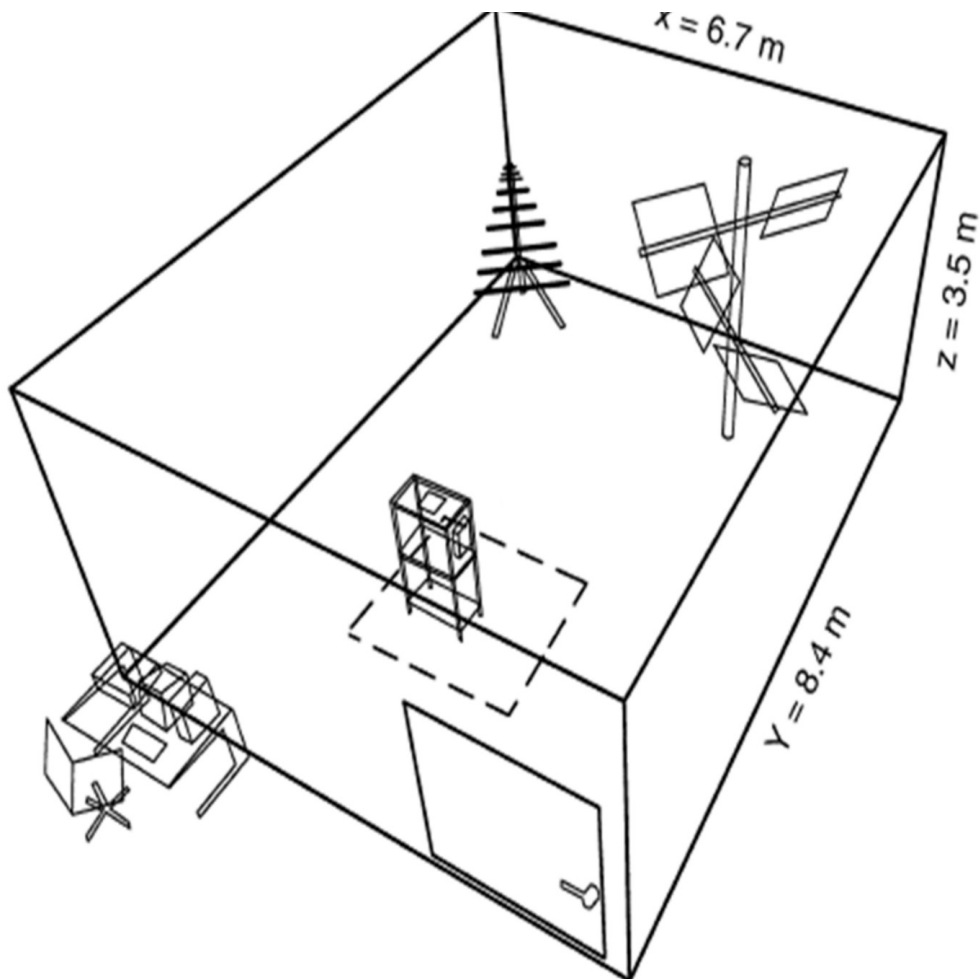
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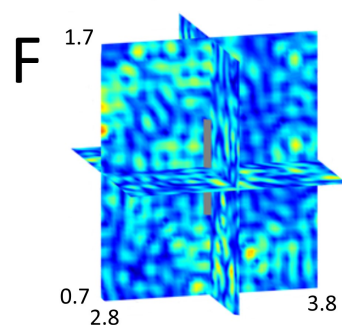
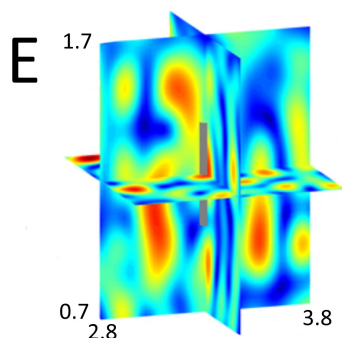
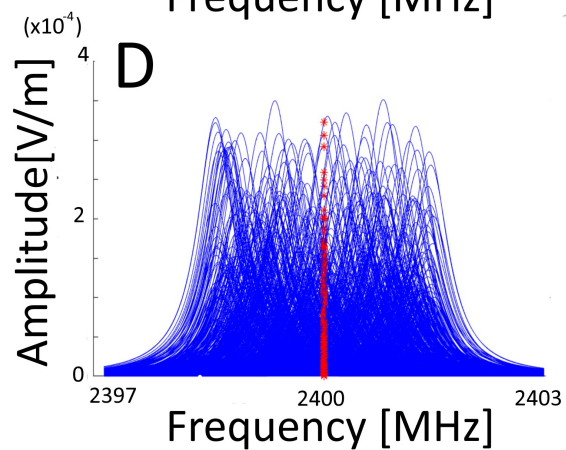
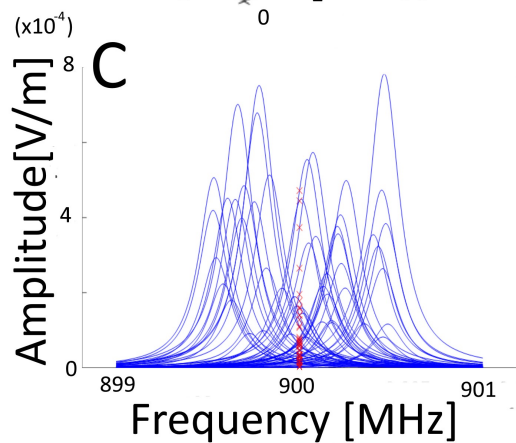
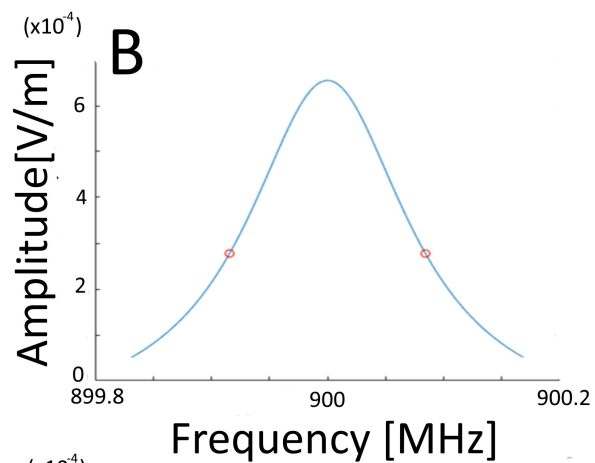
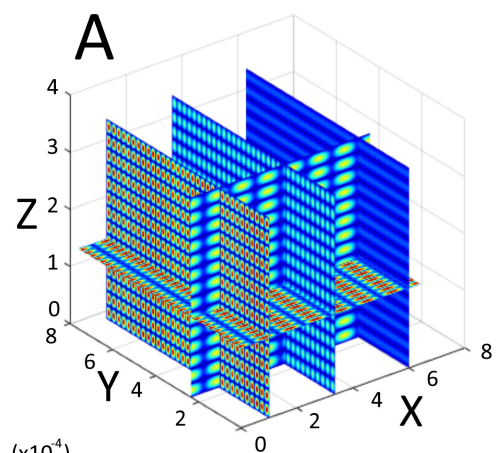
607 **Figures**

608 **Fig. 1:**



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610 **Fig. 2:**



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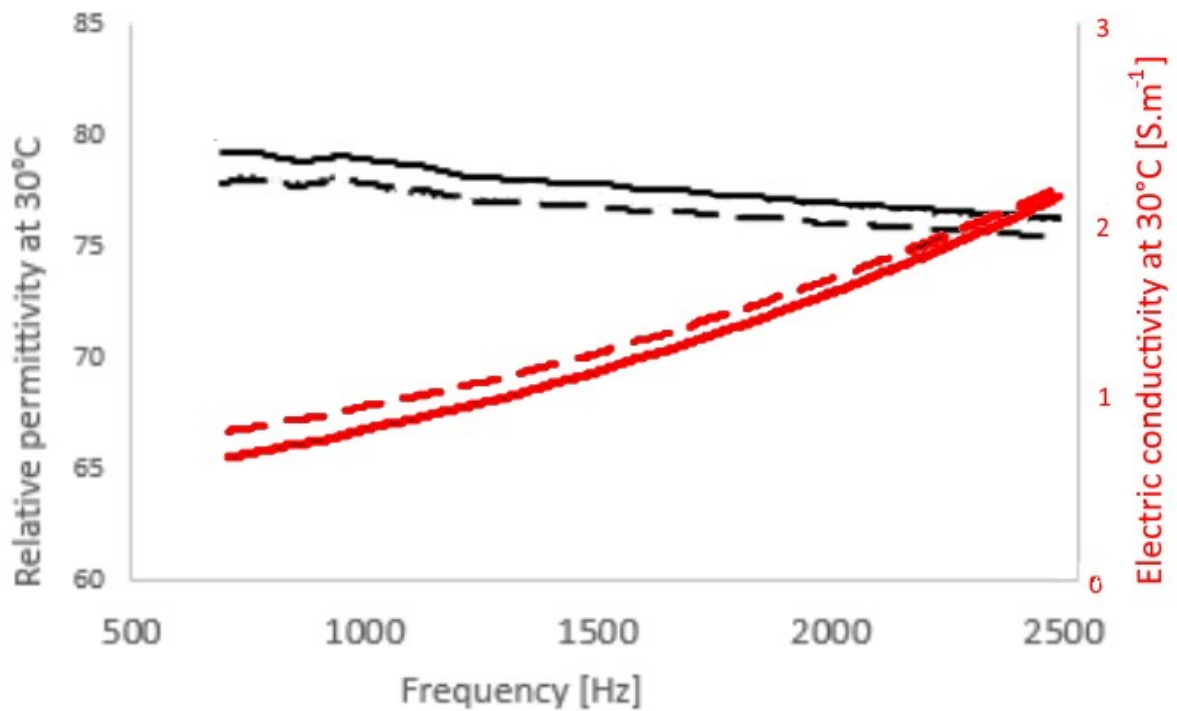
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Bertrand, E., Pasquier, C., Duchez, Girard, Pons, Bonnet, Creuly, Dussap (2018).

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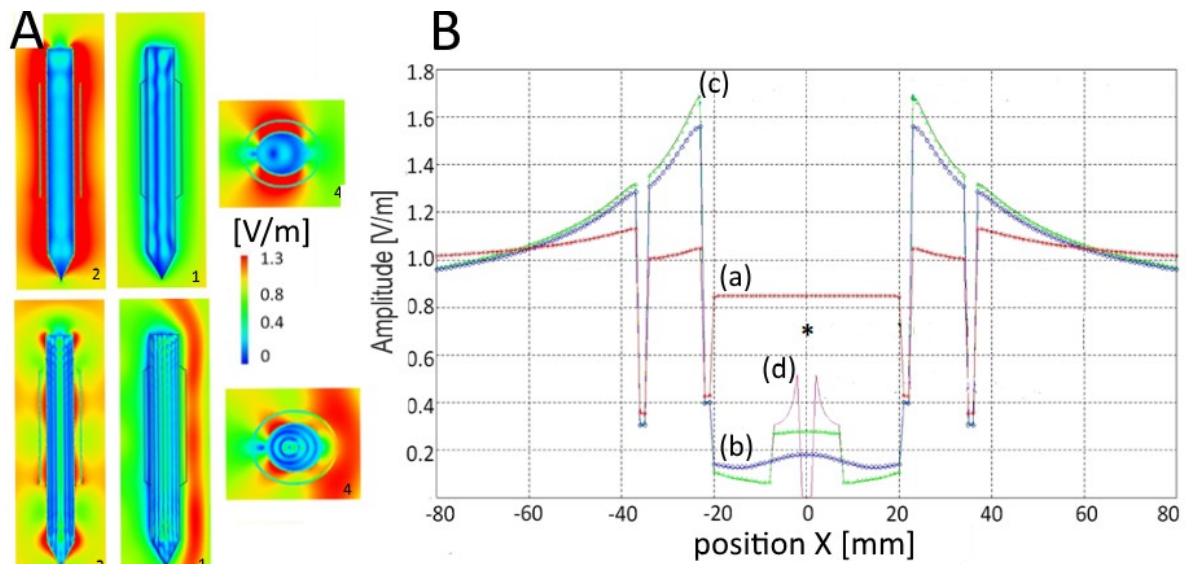
10.1016/i.biortech.2018.03.130

613 Fig. 3:



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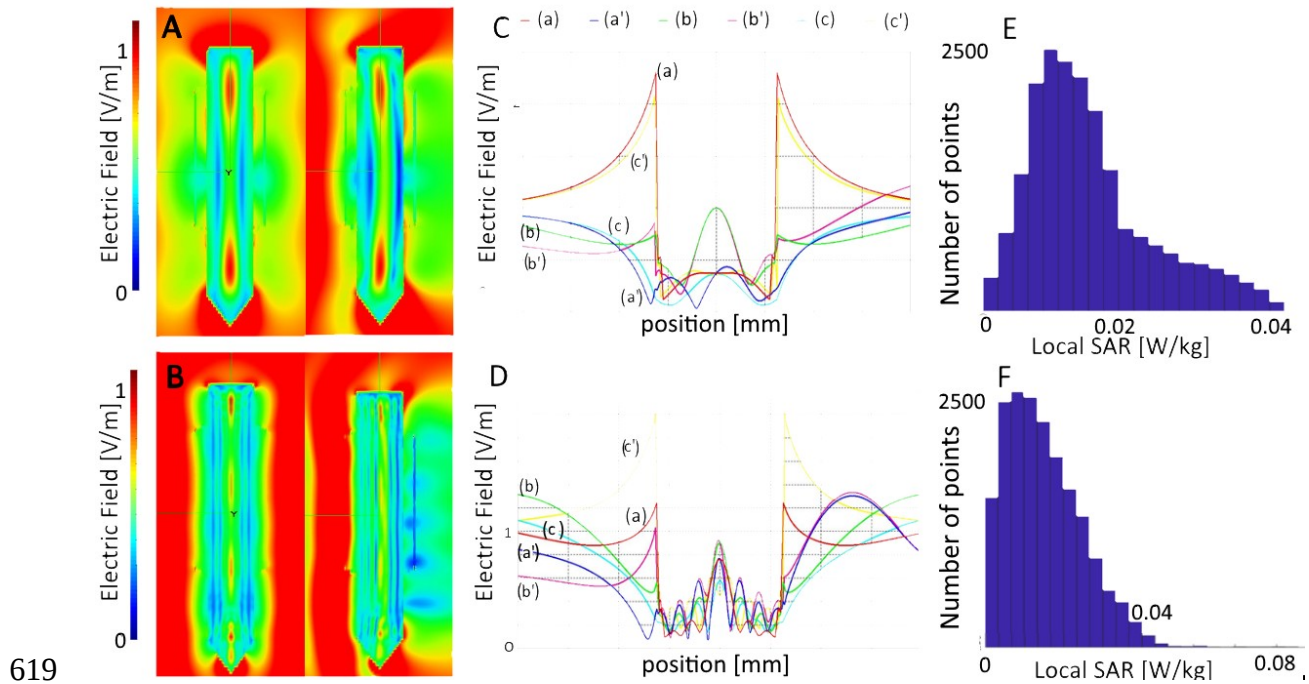
615 Fig. 4:



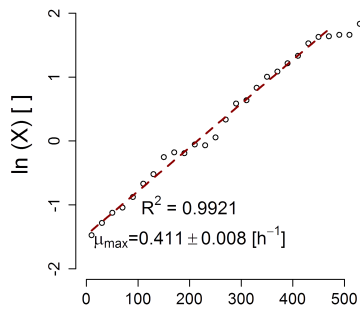
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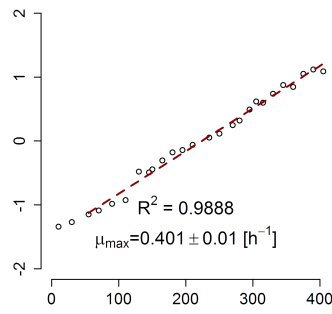
618 Fig. 5:



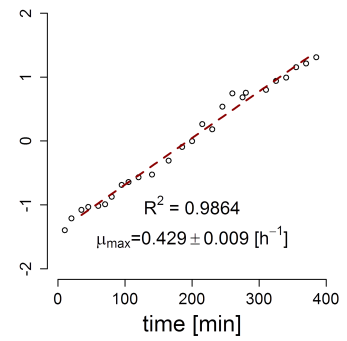
μ_{\max} Référence



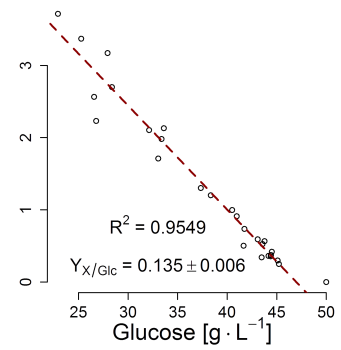
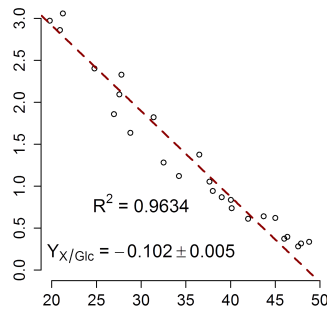
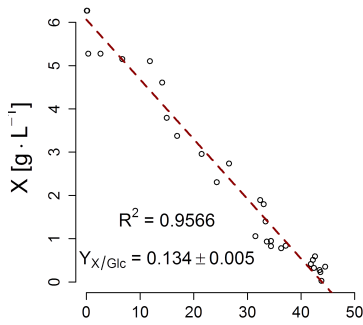
900 MHz



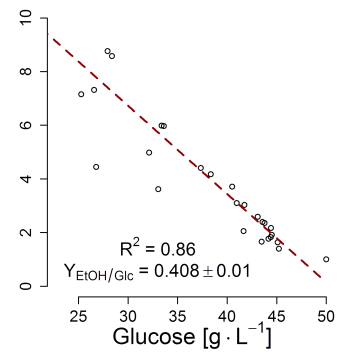
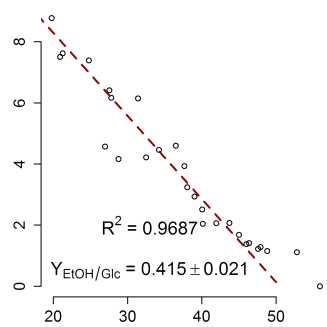
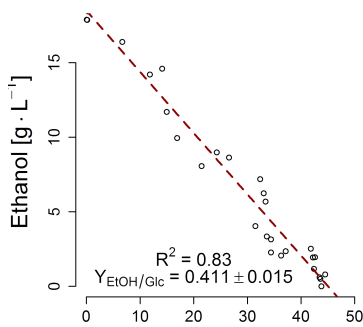
2400 MHz



$Y_{X/\text{Glc}}$



$Y_{\text{EtOH}/\text{Glc}}$



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10.1016/i.biortech.2018.03.130

	Reference	MSRC 900 MHz	MSRC 2400 MHz
[h⁻¹]	0.411 ± 0.008	0.400 ± 0.010	0.429 ± 0.009
	0.134 ± 0.005	0.102 ± 0.048	0.135 ± 0.006
	0.411 ± 0.015	0.415 ± 0.021	0.408 ± 0.010
	0.335 ± 0.011	0.290 ± 0.012	0.305 ± 0.009
	-0.025 ± 0.001	-0.027 ± 0.002	-0.021 ± 0.003
	0.039 ± 0.003	0.016 ± 0.003	0.018 ± 0.003
	0.0070 ± 0.0006	0.0044 ± 0.0019	0.0045 ± 0.0016
	-0.045 ± 0.002	-0.044 ± 0.001	-0.052 ± 0.0023
	-0.0215 ± 0.0005	-0.0074 ± 0.0001	-0.0073 ± 0.0002
	9-13	9-13	9-13
[%]	91.9 ± 3.6	94.6 ± 5.1	89.4 ± 2.8
[%]	74.1 ± 7.2	83.9 ± 5.2	105.3 ± 6.2
[g·L ⁻¹]	0.16 ± 0.1	0.19 ± 0.32	0.5 ± 0.3
Mean Electric Field Inside the Reactor [V.m ⁻¹]		6.1080	3.4431
Average Local Specific Absorption Rate (SAR) [W.kg ⁻¹]	-	0.0175	0.0147
Maximum Local Specific Absorption Rate (SAR) [W.kg ⁻¹]	-	0.0395	0.0794

624 **Figures Legends**

625 **Fig.1:** Schematic representation of the Mode Stirred Reverberation Chamber at Institut Pas-
626 cal.

627

628 **Fig.2: A:** Electric field strength repartition in the MSCR for modes 7/33/14. **B:** MSCR re-
629 sponse at 900 MHz. The red circles represent the bandwidth. **C:** All modes activated in the
630 MSCR for a 900 MHz excitation. **D:** All modes activated in the MSCR for a 2400 MHz excit-
631 ation. **E:** Electric field around the reactor at 900 MHz **F:** Electric field around the reactor at
632 2400 MHz. The reactor is represented in grey at the center of figures E and F.

633

634 **Fig.3:** Measured relative-permittivity (black lines) and electric conductivity (red lines) of the
635 minimal medium before (full line) and after (dotted line) the culture of *Saccharomyces*
636 *cerevisiae* as a function of the frequency. The periodical oscillations are due to the measure-
637 ment system.

638

639 **Fig.4: A:** Profiles comparisons for the simulated electric field inside the secondary reactor il-
640 luminated with a planar wave at 900 MHz (top left) and 2400 MHz (bottom left). Numbers
641 correspond to the cutting plans presented in figure 1B. **B:** Evaluation of the influence of the
642 electromagnetic field probe (and its protection) insertion into the irradiated part of the reactor
643 on the experimental measurements at 900 MHz. (a) simulated electric field inside the empty
644 reactor; (b) simulated electric field inside the culture medium; (c) simulated electric field after
645 the insertion of the protection for the probe; (d) simulated electric field after the insertion of
646 the probe itself; * represents the value experimentally measured by the probe at the center of
647 the secondary reactor.

648 **Fig.5 A:** Free space simulated electric field near the bioreactor at 900 MHz and **B:** 2400
649 MHz. Barographs are voluntarily limited to 0-1 V/m for a better visualization. **C:** Maximum
650 electric field profile inside the bioreactor for the three-different incident polarizations for
651 (XZ): a, b and c and (YZ): a', b', c' plane at 900 MHz and **D:** 2400 MHz (bottom). **E:** Distri-
652 bution of the 20000-calculated local SAR inside the bioreactor at 900 MHz and **F:** 2400 MHz.
653

654 **Fig.6:** Determinations of the maximal growth rate (μ_{\max}), of the biomass to glucose reaction
655 yield (Y_{X-Glc}), and of the ethanol to glucose reaction yield ($Y_{EtOH-Glc}$), using linear regressions
656 assuming the Monod hypothesis.

657

658 **Table Legends**

659 **Table 1:** Maximal growth rate (μ_{\max}) and its standard deviation, Reaction Mass Yields (Y_{X-Glc} :
660 biomass to glucose, $Y_{EtOH-Glc}$: Ethanol to glucose, Y_{Am-Glc} : Ammonium to glucose, Y_{AA-Glc} :
661 Amino acids to glucose, $Y_{Gly-Glc}$: Glycerol to glucose, $Y_{Ace-Glc}$: Acetate to glucose), Respiratory
662 Quotient (RQ mol CO₂/mol O₂), Carbon and Nitrogen Mass Balances (B_C and B_N: percentage
663 of elements recovery considering the products concentration experimental assessment), half
664 velocity constant (K_s : Monod model), Mean electric field inside the reactor, average and
665 maximum Local Specific Absorption Rate (Local SAR) for the selected bioreactor cultures.

666 |