# Influence of food intrinsic factors on the inactivation efficacy of cold atmospheric plasma: impact of osmotic stress, suboptimal pH and food structure

Smet, C[a,b]., Noriega, E.[a,b,1], Rosier, F.[b], Walsh, J.L.[c], Valdramidis, V.P.[d], and

Van Impe, J.F.[a,b]

[a]CPMF[2] – Flemish Cluster Predictive Microbiology in Foods – www.cpmf2.be

[b]BioTeC+ - Chemical and Biochemical Process Technology and Control, Department of Chemical Engineering, KU Leuven, Belgium

[c]Department of Electrical Engineering and Electronics, University of Liverpool, United Kingdom

[d]Department of Food Studies and Environmental Health, Faculty of Health Sciences, University of Malta, Malta

Correspondence to:

Prof. J. F. Van Impe

Chemical and Biochemical Process Technology and Control (BioTeC+)

Department of Chemical Engineering, KU Leuven

Gebroeders De Smetstraat 1, B-9000 Gent (Belgium)

jan.vanimpe@cit.kuleuven.be

Tel: +32-16-32.14.66

Fax: +32-09-265.87.24

---

[1] Author present address: ILSI Europe, Avenue E. Mounier 83, Brussels, Belgium

# ABSTRACT

Cold atmospheric plasma (CAP) has proved to have great potential as a mild food decontamination technology. Different process parameters, including food intrinsic factors, are known to influence the resistance of the cells towards the treatment. The importance of osmotic stress (0, 2, 6% (w/v) NaCl) and suboptimal pH (5.5, 6.5, 7.4) on the CAP efficacy to inactivate *Salmonella* Typhimurium and *Listeria monocytogenes* is studied for various food structures. The helium-oxygen plasma was generated by a dielectric barrier discharge reactor, treating samples up to ten minutes. If grown under osmotic stress or at suboptimal pH, microbial cells adapt and become more resistant during CAP treatment (stress hardening). Additionally, the microorganisms and the food structures also influence the inactivation results. This study illustrates the importance of increasing knowledge on food intrinsic factors, to be able to predict the final CAP inactivation result.

**Keywords:** cold atmospheric gas plasma; osmotic stress; suboptimal pH; food structure; growth morphology.

# 1. INTRODUCTION

Despite all efforts, more than 300,000 food poisonings are still reported every year in the EU (EFSA and ECDC, 2015). Combined with an increasing consumer demand for minimally processed foods, this encourages the development of innovative mild decontamination techniques, like Cold Atmospheric Plasma (CAP). By applying a voltage to a gas stream, the gas atoms or molecules become ionized once the breakdown voltage is exceeded, creating plasma. The plasma phase of matter consists of a mixture of electrons, ions, atomic species, free radicals and UV photons, all able to inactivate microorganisms (Deng, Shi, & Kong, 2006; Perni et al., 2007). The CAP mode of action for inactivation of microbial cells may be explained at different levels (Fernandez & Thompson, 2012; Laroussi & Leipold, 2004; Moisan et al., 2002; Niemira, 2012). Reactive oxygen and nitrogen species interact with macromolecules, like lipids, amino acids and nucleic acids, and cause changes that lead to microbial death or injury. Next to this, charged particles accumulate at the surface of the cell membrane and induce its rupture. In addition, UV photons modify the DNA of the microorganisms. However, Deng et al. (2006) demonstrated that reactive plasma species play a dominant role as compared to the effect of charged particles and UV. When studying the influence of the reactive gas species on the inactivation of *Bacillus subtilis* spores using a helium-(oxygen) plasma, oxygen atoms, metastable oxygen molecules, OH, nitrogen containing species and ozone are responsible for spore inactivation. The low temperature during the CAP treatment, short treatment times, together with the fact that no residues remain on the product after the treatment (Moisan et al., 2001), sum up some of the most important advantages of using CAP for food treatment. However, as plasma is able to adapt bacteria at a cellular level, cells can become sublethally injured as a result from the treatment. This possible disadvantage may pose public health concerns since sublethal injured cells are susceptible to recovery (Noriega, Velliou, Van Derlinden, Mertens, & Van Impe, 2014).

41    Different process parameters influence the efficacy of the plasma treatment. First of all, all

42    plasma characteristics play an important role, like the treatment time, the plasma power, the

43    voltage and frequency applied, together with the gas flow rate and gas composition (Deng et

44    al., 2007; Han, Patil, Keener, Cullen, & Bourke, 2014; Lerouge, Wertheimer, & Yahia, 2001).

45    The CAP set-up itself also has an impact on the decontamination efficacy, as plasma can be

46    produced using, e.g., a plasma jet or a dielectric barrier discharge (DBD) electrode, and a

47    sample can be treated directly or indirectly (Ehlbeck et al., 2011; Fernandez & Thompson,

48    2012; Fridman et al., 2007). Finally, both the microorganism treated and the sample itself,

49    influence the efficacy of the treatment (Afshari & Hosseini, 2012; Fernandez & Thompson,

50    2012). The species of the microorganism, together with its microbial load and the growth

51    phase of the treated cells, play an important role during the CAP inactivation, as reported in

52    literature (Fernandez & Thompson, 2012; Fernandez, Shearer, Wilson, & Thompson, 2012;

53    Lerouge et al., 2001). Preservation of the sample, prior to CAP treatment, also affects the

54    inactivation efficacy. For example, prolonged storage times may trigger the formation of

55    biofilms, resulting in the production of polysaccharide matrices (Giaouris, Chorianopoulos, &

56    Nychas, 2005). These matrices shield the cells against the CAP produced, promoting

57    resistance towards the inactivation treatment (Laroussi, 2009; Vleugels et al., 2005).

58    Regarding the treated sample itself, studies often focus on the CAP inactivation of cells on a

59    specific food product (Fernandez, Noriega, & Thompson, 2013; Gurol, Ekinci, Aslan, &

60    Korachi, 2012; Kim et al., 2011; Selcuk, Oksuz, & Basaran, 2008). General studies taking

61    into account the influence of the (food) sample, or its preservation, on the CAP efficacy are

62    limited.

63    However, food intrinsic factors affect the resistance of the microorganisms towards CAP

64    treatment as well. For example, regarding the influence of the intrinsic food structure of the

65    sample on the CAP efficacy, two different factors can play an important role. First, as a

66 consequence of the food structure, the growth morphology of the cells affects the efficacy of

67 the treatment. Colony immobilization results in cells that are more resistant towards CAP as

68 compared to treatment of cells grown planktonically (Smet et al., 2016). Secondly, the carrier

69 on which cells are deposited during the treatment is important, influencing the CAP efficacy

70 (Lerouge et al., 2001). The interference of plasma species with the cells depends on the type

71 of carrier. In Smet et al. (2016), the CAP inactivation for *S.* Typhimurium and

72 *L. monocytogenes* cells on a liquid carrier, a solid(like) surface and a filter were compared.

73 Cells dispersed inside a liquid carrier are the most difficult to inactivate as most highly

74 reactive plasma species are not able to penetrate the liquid. Additionally, food surfaces can

75 have complex topographies, protecting the cells against plasma-generated species (Fernandez

76 et al., 2013). Next, the acidity of the sample influences the CAP treatment. *S.* Enteritidis cells

77 on agar containing microscope slides were more susceptible towards CAP treatment at pH 5

78 as compared to pH 7 (Kayes et al., 2007). For the same microorganism inactivated in a liquid

79 carrier, an increasing acidity results in a decreasing CAP resistance (Rowan et al., 2007a).

80 In the present work, the role of food intrinsic factors on the efficacy of CAP inactivation is

81 further investigated by focusing on the influence of osmotic stress, in combination with

82 suboptimal pH values. These stresses are respectively represented by growth of *Salmonella*

83 Typhimurium and *Listeria monocytogenes* cells in model systems at different salt

84 concentrations (0, 2, 6% (w/v) NaCl) and pH values (5.5, 6.5, 7.4). Additionally, sublethal

85 injury resulting from CAP treatment is assessed for all experimental conditions.

86

## 2. MATERIALS AND METHODS

87

### 2.1 Experimental plan

88

89 The influence of osmotic stress and suboptimal pH on the CAP efficacy to inactivate

90 *Salmonella* Typhimurium and *Listeria monocytogenes* was studied. Cells were grown under

91  different salt concentrations (0, 2, 6% (w/v) NaCl) and pH values (5.5, 6.5, 7.4). The

92  suboptimal pH range was selected to mimic pH values representing most food products. A

93  broad range of salt levels were examined, also taking into account very salty food products.

94  Additionally, a third intrinsic factor, the influence of the food structure, was studied.  Two

95  growth morphologies, planktonic cells or surface colonies, are a consequence of the intrinsic

96  food structure. Surface colonies were promoted by the addition of 5% (w/v) gelatin to the

97  growth medium, an amount often found in food products (e.g., meat). During inactivation,

98  cells were CAP treated in a liquid carrier or on a solid(like) surface, mimicking treatment of

99  solid and liquid food products. Figure 1 summarizes this experimental plan. As indicated on

100  the figure, different combinations regarding the food structure, thus growth morphology and

101  inactivation support system, were investigated. The logical combinations are (1) cells grown

102  planktonically, followed by inactivation in a liquid carrier and (2) cells grown as surface

103  colonies which are also CAP inactivated on a solid(like) surface. This situation arises when

104  cells have grown in a certain food product, which is treated by CAP. However, two less

105  evident combinations were also studied. First, cells grown planktonically that are inactivated

106  on a solid(like) surface. For example, this situation can appear when microorganisms grow in

107  water used to rinse of dirt from fresh produce, and attach to the food product which is plasma

108  treated. Secondly, cells grown as surface colonies that are inactivated in a liquid carrier. This

109  last scenario holds for cells grown on the surface of the food product, e.g., fresh produce,

110  which is further on in the process blended into a juice and treated with plasma.

111

112  **2.2 Microorganisms and pre-culture conditions**

113  *Salmonella enterica* serovar Typhimurium SL1344 was kindly provided by the Institute of

114  Food Research (IFR, Norwich, UK). The culture was stored at -80°C in Tryptone Soya Broth

115  (TSB (Oxoid LTd., Basingstoke, UK)) supplemented with 25% (v/v) glycerol (Acros

116    Organics, NJ, USA). For every experiment, a fresh purity plate was prepared from the frozen

117    stock culture by spreading a loopful onto a Tryptone Soya Agar plate (TSA (Oxoid Ltd.,

118    Basingstoke, UK)) incubated at 37°C for 24 h. One colony from this plate was transferred into

119    20 mL TSB and incubated under static conditions at 37°C for 8 h (Binder KB series

120    incubator; Binder Inc., NY, USA). Next, 200 μL from this stationary phase culture was added

121    to 20 mL of fresh TSB and incubated under the same conditions for 16 h.

122    *Listeria monocytogenes* LMG 13305 was obtained from the Belgian Co-ordinated Collections

123    of Microorganisms (BCCM, Ghent, Belgium). The culture was stored at -80°C in TSB

124    supplemented with 0.6% (w/v) yeast extract (Merck, Darmstadt, Germany) (TSBYE). For

125    each experiment, a new purity plate was prepared on Brain Heart Infusion (BHI (Oxoid Ltd.,

126    Basingstoke, UK)) supplemented with 1.2% (w/v) agar (Agar technical n°3, Oxoid Ltd.,

127    Basingstoke, UK) and incubated for 24 h at 37°C. One colony from the purity plate was

128    transferred into 20 mL BHI, incubated at 37°C for 8 h under static conditions, refreshed in

129    BHI and incubated again for 16 h.

130    Cell cultivation under the above defined conditions yielded early-stationary phase populations

131    for both *S.* Typhimurium and *L. monocytogenes*, at about $10^9$ CFU/mL. These cultures were

132    used to inoculate the corresponding media at the appropriate concentration.

133

134    **2.3 Growth stage prior to CAP inactivation**

135    During cell growth, the effect of the food structure results in different growth morphologies.

136    Planktonic cells or surface colonies were grown under different experimental conditions of

137    salt concentrations and pH until the early stationary phase was reached. Results from

138    preliminary growth experiments were used to verify this point on the growth curve (Smet,

139    Noriega, Van Mierlo, Valdramidis, & Van Impe, 2015). The preparation and the growth

140    conditions of both the liquid system (planktonic cells) and solid(like) system (surface

141    colonies) are discussed below.

142

143    **2.3.1 Liquid systems: preparation and planktonic growth conditions**

144    For *S.* Typhimurium, the appropriate amount of salt (0, 2, 6% (w/v) NaCl, Sigma Aldrich,

145    MO, USA) was added to TSB without dextrose (Becton, NJ, USA) and the pH (DocuMeter,

146    Sartorius, Goettingen, Germany) was adapted by the addition of 5 M HCl (Acros Organics,

147    NJ, USA). BHI was used for *L. monocytogenes*. Cells were grown in petri dishes (diameter

148    5.5 cm) filled with 7 mL of the medium inoculated at $10^3$ CFU/mL. This cell density was

149    obtained by serial decimal dilutions of stationary phase cells, using dilution medium with the

150    same pH and amount of salt as the final growth conditions. After shaking, the inoculated

151    growth medium was dispensed into petri dishes and placed, under static conditions, in a

152    temperature controlled incubator (KB 8182, Termaks, Bergen, Norway) at 20°C, mimicking

153    the temperature during the CAP treatment. Cells were grown until the early stationary phase

154    was reached.

155

156    **2.3.2 Solid(like) systems: preparation and (surface) colonial growth conditions**

157    Together with the appropriate amount of NaCl, gelatin at 5% (w/v) (gelatin from bovine skin,

158    type B, Sigma-Aldrich, MO, USA) was added to TSB or BHI. After heating for 20 min at

159    60°C in a thermostatic water bath (GR150-S12, Grant Instruments Ltd, Shepreth, UK), the

160    gelatin melted and the medium was adapted to the appropriate pH. The gelled medium was

161    then filter-sterilized using a 0.2 mm filter (Filtertop, 150 mL filter volume, 0.22 μm, TPP,

162    Switzerland), kept liquid at 60°C, and 7 mL was pipetted into sterile petri dishes (diameter 5.5

163    cm), which was left to solidify. Next, the solid(like) plates were surface inoculated at

164    approximately $3.0 \times 10^2$ CFU/cm$^2$ (surface area 23.8 cm$^2$, corresponding to $10^3$ CFU/mL), by

165 using serial decimal dilution of stationary phase cells with the appropriate dilution medium.

166 Following this step, 20 µL of the corresponding dilution was spread onto each petri dish.

167 After being sealed, plates were placed in a temperature controlled incubator at 20°C under

168 static conditions and grown until the early stationary phase was reached.

169

170 **2.4 Sample inoculation for CAP inactivation**

171 Early stationary phase cells were CAP treated as these cells have the highest resistance and

172 are predominantly encountered in a natural environment (Hurst, 1977; Rees, Dodd, Gibson,

173 Booth, & Stewart, 1995).

174 When the planktonic cells or surface colonies reached the early stationary phase, samples

175 were again diluted, using dilution medium with the same NaCl concentration and pH value,

176 and were again inoculated in/on the selected support system. When inactivated in a liquid

177 carrier, the sample was properly diluted (or re-melted in case of surface colonies) to obtain a

178 cell density of 5.5 log(CFU/mL), and 100 µL was pipetted on empty 5 cm petri dishes, which

179 were closed until CAP treatment. Regarding inactivation on a solid(like) surface, the gelled

180 surface was prepared in a 5 cm petri dish (surface area 19.6 cm$^2$), at similar experimental

181 conditions regarding salt level and pH as the initial growth medium. Following this, 50 µL of

182 the appropriately diluted sample of either planktonic or surface colony cells was pipetted and

183 spread on the gelled surface, which was allowed to dry for 40 min in the laminar flow cabinet

184 (Telstar Laboratory Equipment, Woerden, the Netherlands). This results in a final cell density

185 of 5.5 log(CFU/cm$^2$) before inactivation. Cell inoculation on a (membrane) filter (cyclopore

186 PC circles, 0.2 µm, diameter 2.5 cm, Whatman, Maidstone, UK) was identical to the

187 procedure for solid(like) surfaces, except that only 12.5 µL was pipetted and spread on the

188 filter area (4.9 cm$^2$), resulting again in a final inoculum density of 5.5 log(CFU/cm$^2$).

189

190 **2.5 CAP: equipment and inactivation procedure**

191 The dielectric barrier discharge reactor used to study microbial inactivation is illustrated in

192 Figure 2. The discharge was generated between two electrodes (diameter 5.5 cm), covered by

193 a dielectric layer (diameter 7.5 cm). In this set-up, the electrode gap can be varied from 0 to 1

194 cm (fixed at 1 cm in these experiments). An enclosure (22.5 cm x 13.5 cm x 10 cm) around

195 the electrode increases the residence time of the plasma species around the sample while also

196 providing a more controlled environment. The enclosure was not airtight, and so oxygen and

197 nitrogen from the environment were present. The plasma power supply transforms a low

198 voltage DC input (0-60V) into a high voltage AC signal (0-20kV), at a frequency up to 30

199 kHz.

200 The plasma was generated in a gas mixture of helium (purity 99.996%, at a flow rate of 4

201 L/min) and oxygen (purity $\geq$ 99.995%, at a flow rate of 40 mL/min). The two flows were

202 mixed before entering the plasma chamber (total flow rate 4.04 L/min). Thus, a 1% (v/v)

203 admixture of oxygen was added to the helium. For this flow rate, the residence time in the

204 enclosure was approximately 45 s. Due to the use of this helium/oxygen mixture, the key

205 reactive gas species generated are: helium metastables (He*, $He_2$*), atomic oxygen (O),

206 excited atomic oxygen: O(1D), O(1S), exited oxygen: $O_2$(1D), $O_2$(1S), vibrationally excited

207 oxygen: $O_2$(v=1-4), ozone ($O_3$), hydrogen species (H, OH, $HO_2$, $H_2O_2$), nitrogen oxides (NO,

208 $NO_2$, $NO_3$, $N_2O$) and oxygen ions ($O_2^+$, $O_4^+$, $O^-$,$O_2^-$,$O_3^-$, $O_4^-$) (Murakami, Niemi, Gans,

209 O'Connell & Graham, 2014).

210 Samples were placed between the 0.8 cm gap of the DBD electrodes, and after flushing the

211 reactor with the helium-oxygen gas mixture for 4 min, the high-voltage power source was

212 energized and the plasma was generated. Both electrical and optical methods of

213 characterization were employed to ensure a stable plasma discharge. Samples were treated up

214 to 10 minutes (3 min for the filters) at a peak-to-peak voltage around 7 kV, frequency of

215    15 kHz and dissipated plasma power of 9.6 W. For these experimental conditions, the

216    temperature increase of the sample, measured directly after treatment, was about 2°C.

217    Additionally, as was reported in Smet et al. (2016), at longer treatment times ($\geq$ 5 min) the

218    liquid carrier significantly evaporated due to the CAP generated, at a rate of $0.0087 \pm 0.0006$

219    g/sec. Control tests confirmed the evaporation was not due to the gas flow, as for tests

220    executed without any current the sample volume remained intact.

221

222    **2.6 Cell recovery and microbiological analysis**

223    To detect the cell density following CAP inactivation, the viable plate counting technique was

224    used. Following the CAP treatment, some cells might be sublethally injured. Sublethal injury

225    (SI) was defined as a consequence of exposure to a chemical or physical process that damages

226    but does not kill a microorganism (Hurst, 1977). In order to calculate the percentage of

227    sublethal injury (% SI) resulting from CAP treatment, viable plate counting on both general

228    and selective media was performed to determine the cell density after CAP treatment. For

229    cells inactivated in a liquid carrier, 900 µL of saline solution (0.85% (w/v) NaCl) was added

230    to the sample. Afterwards, the diluted sample (1 mL) was collected from the petri dish and

231    transferred to a sterile Eppendorf, in order to prepare serial decimal dilutions. For cells

232    inactivated on the solid(like) surface, the content of the petri dish was transferred to a

233    stomacher bag, liquefied in a thermostatic water bath at 37°C and homogenized in the

234    stomacher for 30 seconds. 1 mL was taken from this bag, and serial decimal dilutions were

235    prepared with saline solution. Regarding inactivation on a filter, the filter was transferred to a

236    stomacher bag containing 5 mL of the saline solution and homogenized in the stomacher for

237    30 seconds. Similarly, 1 mL was pipetted from this bag, to prepare serial decimal dilutions.

238    For each sample, 2-4 dilutions were plated (49.2 µL) onto TSA or BHI-Agar plates (general

239    media) and XLD-Agar (*S*. Typhimurium, Xylose Lysine Deoxycholate Agar, Merck & Co,

240   New Jersey, USA) or PALCAM-Agar (*L. monocytogenes*, VWR Chemicals, Leuven,

241   Belgium) plates (selective media) using a spiral plater (Eddy-Jet, IUL Instruments). Plates

242   with general media were placed at 37°C for 24 h before counting, while selective plates were

243   stored up to 48 h at 30°C. Cell counts shown in the figures are the mean of all countable

244   dilutions for each sample.

245

246   **2.7 Modelling, parameter estimation and estimation of sublethal injury**

247   The model of Geeraerd, Herremans and Van Impe (2000), was used to fit experimental data.

248   This model describes a microbial inactivation curve consisting of a shoulder, a loglinear

249   inactivation phase and a tail:

250   $$N(t) = (N_0 - N_{res}) \cdot \exp(-k_{max} \cdot t) \cdot \left( \frac{\exp(k_{max}.t_l)}{1+(\exp(k_{max}.t_l)-1)\cdot\exp(-k_{max}\cdot t)} \right) + N_{res} \qquad (1)$$

251   with $N(t)$ [CFU/mL] the cell density at time $t$ [s], $N_0$ [CFU/mL] the initial cell density,

252   $N_{res}$ [CFU/mL] a more resistant subpopulation, $k_{max}$ [1/s] the maximum specific inactivation

253   rate and $t_l$ [s] the length of the shoulder. The regression analysis was performed using the log

254   transformation of Equation 1. The final *log reduction* is calculated from the difference

255   between log $N_0$ *and* log $N_{res}$, using log $N(t=600 sec)(or$ log $N(t=180 sec)$, for inactivation on a

256   filter) if log $N_{res}$ was not yet reached.

257   Parameters of the Geeraerd et al. (2000) model were estimated via the minimization of the

258   sum of square errors (SSE), using the *lsqnonlin* routine of the Optimization Toolbox of

259   Matlab (The Mathworks Inc.). Simultaneous with parameter estimation, the parameter

260   estimation errors were determined based on the Jacobian matrix. The Root Mean Squared

261   Error (RMSE) was added as an absolute measure of the goodness of the model fit to the actual

262   observed data.

263   In order to calculate the percentage of sublethal injury (% SI), theoretical concentrations

264   obtained from the model were used. The percentage of injured survivors after exposure to

265 CAP treatment was determined using the following equation (Busch & Donnelly, 1992),

266 providing the extent of the injured population at each exposure time, and the obtained values

267 were used to simulate the percentage of sublethal injury with respect to treatment time:

268 $\% \text{ Sublethal Injury} = \frac{\text{counts on non selective medium} - \text{counts on selective medium}}{\text{counts on non selective medium}} \cdot 100$ (2)

269

270 **2.8 Statistical analysis**

271 Analysis of variance (ANOVA) test was performed to determine whether there were

272 significant differences amongst means of logarithmically transformed viable counts, at a

273 95.0% confidence level ($\alpha = 0.05$). The Fisher´s Least Significant Difference (LSD) test was

274 used to distinguish which means were significantly different from which others. Standardized

275 skewness and standardized kurtosis were used to assess if data sets came from normal

276 distributions. These analyses were performed using Statgraphics Centurion XVI.I Package

277 (Statistical Graphics, Washington, USA). Test statistics were regarded as significant when P

278 was $\leq 0.05$.

279

280 # 3. RESULTS AND DISCUSSION

281 Figure 3 and 4 represent the inactivation curves of stationary phase *S.* Typhimurium and

282 *L. monocytogenes* cells exposed to CAP treatment. For each microorganism, cells were

283 inactivated in a liquid carrier (a, b, c), on a solid(like) surface (d, e, f) or on a filter (g, h, i).

284 Prior to the CAP treatment, cells were grown at three different experimental conditions, in

285 order to assess the influence of osmotic stress in combination with a suboptimal pH: pH 7.4,

286 0% (w/v) NaCl (a, d, g), pH 6.5, 2% (w/v) NaCl (b, e, h) or pH 5.5, 6% (w/v) NaCl (c, f, i).

287 Additionally, different food structures of the model system result in cells grown

288 planktonically or as surface colonies. As a control, for the most optimal experimental

289    condition at pH 7.4 and 0% (w/v) NaCl (a, d, g), cells from the pre-culture were also directly

290    treated. The experimental data were fitted with the Geeraerd et al. (2000) model. Table 1 and

291    2 summarize the estimated main inactivation parameters from the Geeraerd model, i.e., the

292    length of the shoulder phase $t_l$, the inactivation rate $k_{max}$, the cell density in the tail log $N_{res}$, the

293    overall *log reduction* and the corresponding statistical analysis.

294

295    Regarding the response of both microorganisms to CAP exposure, under optimal

296    experimental conditions inactivation kinetics of the Gram-negative *S.* Typhimurium and

297    Gram-positive *L. monocytogenes* have similar shapes. However, *L. monocytogenes* is more

298    resistant to the CAP treatment, resulting in the observation of lower inactivation efficacies. As

299    previously discussed by Smet et al. (2016), the diversity in the CAP efficacy can be explained

300    by the different cell wall structures of the two microorganisms. Gram-positive bacteria are

301    often found to be more resistant towards CAP treatment than Gram-negative bacteria

302    (Ermolaeva et al., 2011; Lee, Paek, Ju, & Lee., 2006). The addition of osmotic stress or

303    suboptimal pH to the cell environment does not change this observation, as also at pH 6.5, 2%

304    (w/v) NaCl (b, e, h) and pH 5.5, 6% (w/v) NaCl (c, f, i) *L. monocytogenes* has a lower

305    inactivation efficacy as compared to *S.* Typhimurium (Figure 3 and 4).

306

307    **3.1 Effect of osmotic stress and suboptimal pH on CAP inactivation efficacy**

308    *3.1.1 Effect of osmotic stress and suboptimal pH for cells inactivated in a liquid carrier*

309    For all experimental conditions, the inactivation curves of *S.* Typhimurium cells treated in a

310    liquid carrier (a, b, c) present a long shoulder phase followed by a log linear inactivation

311    phase, regardless their growth morphology. This long shoulder phase reflects a resistant

312    population and implies that the microorganisms need to have a certain CAP treatment level

313    before the cells are lethally damaged. As the stress level rises due to an increase of the salt

314    level in combination with a decrease of the pH value, the reduction in cell density due to CAP

315    treatment reduces. This trend is very significant when comparing kinetics for cells grown

316    under optimal conditions (pH 7.4, 0% (w/v) NaCl (a)) with the inactivation dynamics of cells

317    grown under high environmental stress (pH 5.5, 6% (w/v) NaCl (c)). Regarding the

318    inactivation parameters for *S.* Typhimurium, no significant differences are observed for $t_l$

319    values between the three experimental conditions when the cells are grown planktonically.

320    For cells grown as surface colonies, the length of the shoulder phase increases under high

321    osmotic and acidic stresses. The inactivation rate, $k_{max}$, tends to decrease when the

322    environmental stress level increases, irrespective of the growth morphology. No tailing phase

323    is present for cells inactivated in a liquid carrier, thus log $N_{res}$ is undefined. Finally, *log*

324    *reductions* for cells inactivated in a liquid carrier tend to be the lowest at pH 5.5, 6% (w/v)

325    NaCl (c), which is valid for both planktonic cells and surface colonies.

326    In case of *L. monocytogenes*, most survival curves exhibit again a long shoulder phase

327    followed by the log linear inactivation. In all cases, the final reduction is limited, especially in

328    comparison to results for *S.* Typhimurium. Especially at the most stressing condition, pH 5.5

329    and 6% (w/v) NaCl, almost no reduction in cell concentration is observed. No shoulder is

330    present in the kinetics at pH 5.5, 6% (w/v) NaCl (c) for both growth morphologies, or at pH

331    7.4, 0% (a) NaCl for planktonic cells. Therefore, no conclusion can be made regarding the

332    influence of osmotic stress and suboptimal pH on the inactivation parameter $t_l$ for

333    *L. monocytogenes* cells inactivated in a liquid carrier (a, b, c). Similar to the results for

334    *S.* Typhimurium and regardless the growth morphology of the *L. monocytogenes* cells, $k_{max}$

335    and the *log reduction* are the lowest for more stressing experimental conditions, while $N_{res}$ is

336    undefined.

337

338    *3.1.2 Effect of osmotic stress and suboptimal pH for cells inactivated on a solid(like) surface*

339    For *S.* Typhimurium cells inactivated on a solid(like) surface (d, e, f), a shoulder phase is

340    detected for low stress levels (pH 7.4, 0% (w/v) NaCl (d) and pH 6.5, 2% (w/v) NaCl (e)).

341    Following the log linear inactivation phase, sometimes also a tailing phase is present,

342    indicating the presence of a CAP resistant population. This tail was again observed for all

343    growth morphologies at the optimal experimental condition (d), and for planktonic cells at the

344    most severe environmental stresses (e, f). Although no significant differences are present, the

345    inactivation rate decreases slightly when the stress level increases, which is observed for all

346    growth morphologies. A tailing phase is often observed for cells inactivated on a solid(like)

347    surface, but no general trend for $N_{res}$ concerning the influence of the pH value or salt

348    concentration is found. Concerning the influences of osmotic stress and a suboptimal pH on

349    the *log reductions* for *S.* Typhimurium cells inactivated on a solid(like) surface, the reduction

350    tends to be the highest at optimal conditions (d). As the shoulder was not detected for all

351    experimental cases, no conclusion regarding its length can be drawn.

352    Inactivation kinetics for *L. monocytogenes* cells inactivated on a solid(like) surface follow a

353    similar trend (d, e, f). While a shoulder is never observed, and thus $t_l$ is undefined, the linear

354    inactivation phase is always followed by a long tail. As for *S.* Typhimurium, $k_{max}$ values of

355    *L. monocytogenes* decrease when environmental stresses increase, while $N_{res}$ values tend to

356    increase at high osmotic and acidic stresses. Also at pH 5.5, 6% (w/v) NaCl (f), *log reductions*

357    are lower.

358

359    *3.1.3 Effect of osmotic stress and suboptimal pH for cells inactivated on a filter*

360    For *S.* Typhimurium cells inactivated on a filter (g, h, i), the inactivation kinetics do not

361    follow any specific trend. A shoulder phase is only observed for low (pH 7.4, 0% (w/v) NaCl

362    (g)) and medium stress levels (pH 6.5, 2% (w/v) NaCl (h)), so no general conclusion

363    regarding the length of this shoulder can be drawn. Again, $k_{max}$ values for both growth

364    morphologies decrease slightly when the stress level increases. $N_{res}$ is undefined as most

365    conditions do not have a tail. Again, *log reductions* tend to be the lowest at pH 5.5, 6% (w/v)

366    NaCl (i).

367    When *L. monocytogenes* cells are inactivated on a filter (g, h, i), a tail is always observed

368    while the shoulder phase is mainly present at high stress levels. Regarding the inactivation

369    parameters, $t_l$ is often undefined. The decrease in *log reduction* and $k_{max}$ with increasing

370    environmental stress is very limited, and regarding the inactivation rate no significant

371    differences are observed. The tailing phase is always present, and $N_{res}$ slightly increases with

372    an increase of the environmental stress.

373

374    Growth conditions or intrinsic factors do not only influence microbial growth, but are also

375    able to affect the stress response of microorganisms towards CAP treatment. Regardless the

376    inactivation support or the growth morphology, the more stressing the growth conditions

377    concerning pH value and NaCl concentration, the more resistant the microorganisms are

378    towards CAP treatment, resulting in lower inactivation efficacies. For example, if a

379    microorganism would be able to grow in a salty food product (e.g., cheese), CAP treatment

380    might not be sufficient to ensure the food safety. Many bacteria interpret osmotic stress as a

381    signal to prepare for more stringent conditions in the future by inducing a general system of

382    stress protection (O'Byrne & Booth, 2002). Similarly, acid-adapted cells were found to have

383    increased tolerance towards various stresses, including thermal and osmotic stress (Leyer &

384    Johnson, 1993). As a non-thermal technology, CAP is the optimal choice to treat (acid) fruits.

385    However, acid adaption of cells raises problems when the CAP treatment is not able to

386    inactivate them. These stress related phenomena can be explained by cross protection or stress

387    hardening, which refers to an increased resistance to lethal factors, e.g. CAP, after adaptation

388  to environmental stresses (Lou & Yousef, 1997). More specifically, the adaptation to different

389  environmental stresses (acid, ethanol, $H_2O_2$, heat, NaCl) was reported to increase the

390  resistance of *L. monocytogenes* to hydrogen peroxide. This can be explained by the induction

391  of a sigma factor, accounting for the general resistance to environmental stresses in microbial

392  cells (Wesche, Gurtler, Marks, & Reyser, 2009).

393  Limited research is available on the influence of intrinsic or extrinsic factors on the CAP

394  efficacy. Fernandez et al. (2013) studied the effect of the growth temperature on the CAP

395  inactivation of *S.* Typhimurium. In the observed range of temperatures from 20°C to 45°C,

396  the growth temperature did not significantly affect the resistance of the microorganism

397  towards the CAP treatment. However, an increased resistance to CAP treatment, after

398  adaptation to environmental stress has been previously reported. For example, in Smet et al.

399  (2016), the influence of the intrinsic food structure on the CAP efficacy to inactivate

400  *S.* Typhimurium and *L. monocytogenes* was studied. During bacterial growth, different growth

401  morphologies arise as a direct consequence of the intrinsic food structure. As reported, the

402  type of growth morphology influences the CAP efficacy. CAP inactivation experiments with

403  cells grown as surface colonies result in lower log reductions as compared to experiments

404  with planktonic cells, indicating an increased resistance of the surface colonies towards CAP.

405  Starvation stress, resulting from nutrient limitations which surface colonies endure, can create

406  cells resistant to the subsequent CAP inactivation treatment (Li, Sakai, Watanabe, Hotta, &

407  Wachi, 2013). Similar to stress due to the intrinsic food structure or growth temperature, cross

408  protection plays an important role on the CAP inactivation efficacy if cells are grown under

409  osmotic stress or at suboptimal pH values. All environmental stresses, due to the pH, salt

410  level, food structure or growth temperature can result in an increased resistance towards a

411  subsequent CAP treatment.

412

413 **3.2 Effect of food structure on CAP inactivation efficacy for environmental conditions**

414 **under osmotic stress at suboptimal pH**

415 The food structure can affect the CAP inactivation on two different levels. The growth

416 morphology of the cells, a direct consequence of the intrinsic food structure, influences the

417 CAP inactivation efficacy. Secondly, the food structure plays an important role during the

418 treatment itself, by means of the inactivation support system in/on which the cells are

419 deposited.

420 In most cases, these two levels regarding food structure are related. A liquid food product

421 (e.g., a juice) where cells have grown planktonically, can be treated directly and thus the cells

422 are also treated in a liquid carrier. This holds as well if the inactivation support is a solid(like)

423 surface (e.g., meat, fruits, vegetables) infected with surface colonies. However, due to food

424 processing, exceptions can arise. For example, planktonic cells can grow in the washing water

425 used during processing. This could result in cross-contamination onto the food products,

426 which are afterwards treated with CAP. Similarly, all surfaces that come into contact with a

427 (liquid or solid(like)) food product, form a potential risk for contamination if infected with

428 colonies. Finally, if colonies have grown on a the surface of a fruit or vegetable, and this

429 product is blended into a juice, the resulting fruit or vegetable juice itself can be treated with

430 CAP (liquid carrier).

431 This section discusses, on both levels, the effect of the food structure on the CAP inactivation

432 efficacy for the different (stressing) experimental conditions.

433

434 *3.2.1 Effect of the growth morphology on the CAP inactivation efficacy*

435 In Smet et al. (2016), the influence of the food structure on the CAP efficacy was examined

436 under optimal experimental conditions regarding osmotic and acidic stress (pH 7.4, 0% (w/v)

437 NaCl (a, d, g)). The influence of food structure during growth was studied by investigating the

438 role of the growth morphology in the CAP inactivation efficacy, indicating cells were grown

439 planktonically in a liquid environment or as surface colonies on a solid(like) surface. Lower

440 inactivation efficacies for cells grown as surface colonies at pH 7.4, 0% (w/v) NaCl (a, d, g),

441 regardless of the inactivation support system, indicate an increased resistance of these

442 immobilized cells towards CAP inactivation of both *S.* Typhimurium and *L. monocytogenes*.

443 This conclusion still holds when the environmental stress is more severe (Figure 3 and 4). If

444 cells of both microorganisms are inactivated in a liquid carrier and grown at pH 6.5, 2% (w/v)

445 NaCl (b) or pH 5.5, 6% (w/v) NaCl (c), the CAP inactivation is again the highest for

446 planktonic cells, although in some cases the inactivation parameters do not indicate

447 statistically significant differences. In a stressing environment, cells inactivated on the

448 solid(like) surface exhibit lower *log reductions* when grown as surface colonies as compared

449 to cells grown planktonically. Next to this, the inactivation rate follows the order $k_{max,\ planktonic}$

450 $_{cells} \geq k_{max,\ surface\ colonies}$ while log $N_{res}$ is always lower for cells grown planktonically. A similar

451 behavior was detected for cells grown under stressing conditions and CAP treated on a filter.

452 In a solid(like) environment growth takes place as (surface) colonies and the transport is

453 based on diffusion, limiting among others the nutrient delivery (Antwi et al., 2006; Malakar et

454 al., 2000; Wimpenny & Coombs, 1983). This nutrient limitation results in starvation stress,

455 indicating the survival of bacteria in oligotrophic conditions (Wesche et al., 2009), which can

456 promote the resistance of the cells against the subsequent CAP treatment (Li et al., 2013).

457

458 *3.2.2 Effect of the inactivation support system on the CAP inactivation efficacy*

459 The effect of the food structure during the CAP treatments is studied in Smet et al. (2016) by

460 assessing the kinetics of cells, grown under optimal experimental conditions (pH 7.4, 0%

461 (w/v) NaCl (a, d, g)). In the current work cells are inactivated on three different inactivation

462 support systems: a liquid carrier, a solid(like) surface or a filter. Regardless if *S.* Typhimurium

463    or *L. monocytogenes* cells are grown under optimal environmental conditions (a, d, g), or at

464    more stressing pH values and salt levels (b, c, e, f, h, i), the effect of the intrinsic food

465    structure during the treatment on the CAP efficacy remains the same (Figure 3, 4). At optimal

466    environmental conditions, the lack of a shoulder phase for cells inactivated on a solid(like)

467    surface or on a filter leads to a very rapid inactivation as compared to cells inactivated inside a

468    liquid carrier. These different shapes in survival curves are always observed for the different

469    inactivation support systems, independent of the environmental growth condition. Regarding

470    the inactivation parameters, as expected $k_{max}$ values are either similar or slightly lower for

471    cells inactivated in a liquid carrier, grown at pH 6.5, 2% (w/v) NaCl (b, e, h) or pH 5.5, 6%

472    (w/v) NaCl (c, f, i). Therefore, also under more severe environmental stress, cells in a liquid

473    carrier prove to be more difficult to inactivate. As many highly reactive plasma species

474    already react at the plasma-liquid interface and do not penetrate very deep into the liquid

475    medium, cells in a liquid carrier are more challenging to inactivate. Cells treated on a

476    solid(like) surface or on a filter are easily attained by the plasma species during the treatment,

477    resulting in a higher inactivation efficacy (Oehmigen et al., 2010). As commented in Section

478    2.5, the liquid carrier partly evaporates at longer treatment times (≥ 5 min). This could

479    possibly result in a shift of treatment of cells on the plastic petri dish instead of in the liquid

480    carrier which can contribute to the fact that inactivation of cells in the liquid carrier only starts

481    at longer treatment times.

482

483    As previously mentioned, food products containing high salt concentrations or products with

484    lower pH values might not be well suited to be CAP treated as cells could be able to adapt to

485    these environmental stresses and gain resistance towards subsequent CAP treatment. The

486    above findings regarding the influence of the food structure indicate this effect might be

487    magnified if cells are either grown as surface colonies or inactivated in a liquid carrier.

488

### 3.3 CAP and sublethal injury of cells: effect of osmotic and acidic stress

As sublethal injured cells are able to recover or even gain resistance, they pose major public

health concerns. Thus it is important to investigate the relation between novel inactivation

technologies and SI of cells (Noriega et al., 2014). Sublethal injury of cells treated with CAP

was studied by plating the treated cells on both general and selective plating media. The

percentage of sublethal injury (SI) as a function of treatment time is illustrated in Figure 5 and

6.

Most studies focus on enumerating the microbial survivors on general media while limited

research has been performed focusing on the sublethal injury of cells following CAP

treatment. By using respiratory staining (RS), Rowan et al. (2007b) proved the existence and

rapidly quantified the extent of sublethal injury for CAP treated pathogens. As discussed in

Smet et al. (2016), under optimal conditions (pH 7.4, 0% (w/v) NaCl) the SI of

*S.* Typhimurium cells is higher than for *L. monocytogenes*. This trend can be extended to the

more stressing experimental conditions at pH 6.5, 2% (w/v) NaCl (b, e, h) and pH 5.5, 6%

(w/v) NaCl (c, f, i), as the SI detected for *L. monocytogenes* will always be the lowest.

Regardless the environmental growth condition, a maximum for the SI evolution in time is

often detected. This maximum illustrates the phenomenon of injury accumulation finally

culminating into cell death (Noriega, Velliou, Van Derlinden, Mertens, & Van Impe., 2013),

and coincides to the start of a new phase in the inactivation kinetics. As for most

*L. monocytogenes* experiments the kinetics show a tailing phase, the maximum in the SI

evolution corresponds to the transition into this last phase. In case of *S.* Typhimurium, a

maximum is detected at optimal conditions (a, d, g) or moderately stressing environmental

conditions (pH 6.5, 2% (w/v) NaCl (b, e, h)). Also for this microorganism, the maximum

coincides with the transition to either the linear inactivation phase or the tailing phase, if

513    present. However, at more stressing environmental conditions, different trends are sometimes

514    observed, and the evolution of SI with treatment time is still increasing. This holds for

515    *S.* Typhimurium at the most stressing condition (c, f, i), as for these experiments no tail is

516    (yet) reached. Finally, for experiments with a limited overall reduction, the SI evolution with

517    treatment time remains constant

518    As reported in Smet et al. (2016), no trend regarding the influence of the food structure on the

519    SI evolution after CAP treatment is present, which is again valid in a stressed environment.

520    However, there is a direct influence of the osmotic stress and suboptimal pH on the SI as a

521    function of the CAP treatment time. In general, if the stress level increases due to prior

522    growth at high salt concentrations and low pH values, also the level of SI during CAP

523    inactivation increases. For instance, for *S.* Typhimurium at pH 5.5, 6% (w/v) NaCl (c, f, i),

524    some cells are even sublethally injured prior to the CAP treatment, which can be explained

525    due to the high salt concentrations present in the media during growth.

526

## 527    4. CONCLUSION

528    The role of food intrinsic factors on the efficacy of CAP inactivation is further investigated by

529    focusing on the influence of osmotic stress and suboptimal pH on the inactivation kinetics of

530    *S.* Typhimurium and *L. monocytogenes*. The presence of high salt concentrations in the

531    growth medium or suboptimal pH values, induces stress hardening, creating cells resistant

532    towards the subsequent CAP treatment. Additionally, regardless the osmotic stress level or the

533    pH value in the system, both the type of microorganism and the food structure remain to

534    influence the inactivation results. The maximum in the SI evolution as a function of the

535    treatment time, indicates an injury accumulation of the treated cells that finally culminates

536    into cell death. This research again confirms that food intrinsic factors, influence the CAP

537    inactivation efficacy. This indicates the importance of knowledge on the different food

538    intrinsic factors or thus the food properties, e.g., regarding salt concentration, pH value or

539    intrinsic food structure, to be able to predict the final CAP inactivation result. This knowledge

540    makes it possible to assess whether or not CAP can be an efficient mild technology to treat a

541    specific food product.

542

## ACKNOWLEDGEMENTS

553

## REFERENCES

555    Afshari, R., & Hosseini, H. (2012). 2012 Atmospheric Pressure Plasma Technology: A New

556    Tool for Food Preservation. *International Conference on Environment, Energy and*

557    *Biotechnology*, 33, 275-278.

558    Antwi, M., Geeraerd, A. H., Vereecken, K. M., Jenné , R., Bernaerts, K., & Van Impe, J. F.

559    (2006). Influence of a gel microstructure as modified by gelatin concentration on *Listeria*

560    *innocua* growth. *Innovative Food Science & Emerging Technologies*, 7, 124–131.

561    Busch, S. V., & Donnelly, C. W. (1992). Development of a repair-enrichment broth for

562  resuscitation of heat-injured *Listeria monocytogenes* and *Listeria innocua. Applied and*

563  *Environmental Microbiology*, *58*, 14-20.

564  Deng, X., Shi, J. J., & Kong, M. G. (2006). Physical mechanisms of inactivation of *Bacillus*

565  *subtilis* spores using cold atmospheric plasmas. *IEEE Transactions on Plasma Science*, *344*,

566  1310-1316.

567  Deng, S., Ruan, R., Kyoonmok, C., Huang, G., Lin, X., & Chen, P. (2007). Inactivation of

568  *Escherichia coli* on Almonds Using Nonthermal Plasma. *Journal of food science*, 72, M62-

569  M66.

570  Ehlbeck, J., Schnabel, U., Polak, M., Winter, J., von Woedtke, Th., Brandenburg, R., von dem

571  Hagen, T., & Weltmann, K-D. (2011). Low temperature atmospheric pressure plasma sources

572  for microbial decontamination. *Journal of Physics D: Applied Physics*, *44*, 013002.

573  Ermolaeva, S. A., Varfolomeev, A. F, Chernukha, M. Y., Yurov, D. S., Vasiliev, M. M.,

574  Kaminskaya, A. A., Moisenovich, M. M., Romanova, J. M., Murashev, A. N., Selezneva, I. I.,

575  Shimizu, T., Sysolyatina, E. V., Shaginyan, I. A., Petrov, O. F, Mayevsky, E. I., Fortov, V. E.,

576  Morfill, G. E., Naroditsky, B. S., & Gintsburg, A.L. (2011). Bactericidal effects of non-

577  thermal argon plasma *in vitro*, in biofilms and in the animal model of infected wounds.

578  *Journal of Medical Microbiology*, 60, 75–83.

579  European Food Safety Authority (EFSA), & European Centre for Disease Prevention and

580  Control (ECDC) (2015). The European Union summary report on trends and sources of

581  zoonoses, zoonotic agents and food-borne outbreaks in 2013. *EFSA Journal*, *13*:3991.

582  Fernandez, A., & Thompson, A. (2012). The inactivation of *Salmonella* by cold atmospheric

583  plasma treatment. *Food Research International*, *45*, 678-684.

584 Fernandez, A., Shearer, N., Wilson, D. R., & Thompson, A. (2012). Effect of microbial

585 loading on the efficiency of cold atmospheric gas plasma inactivation of *Salmonella enterica*

586 serovar Typhimurium. International Journal of Food Microbiology, 152, 175–180.

587 Fernandez, A., Noriega, E., & Thompson, A. (2013). Inactivation of *Salmonella enterica*

588 serovar Typhimurium on fresh produce by cold atmospheric gas plasma technology. *Food*

589 *Microbiology*, *33*, 24-29.

590 Fridman, G., Brooks, A. D., Balasubramanian, M., Fridman, A., Gutsol, A., Vasilets, V. N.,

591 Ayan, H., & Friedman, G. (2007). Comparison of direct and indirect effects of non-thermal

592 atmospheric pressure plasma on bacteria. *Plasma Processes and Polymers*, *4*, 370–375.

593 Geeraerd, A., Herremans, C., & Van Impe, J. F. (2000). Structural model requirements to

594 describe microbial inactivation during a mild heat treatment. *International Journal of Food*

595 *Microbiology*, *59*, 185-209.

596 Giaouris, E., Chorianopoulos, N., & Nychas, G. J. E. (2005). Effect of temperature, pH, and

597 water activity on biofilm formation by *Salmonella enterica* Enteritidis PT4 on stainless steel

598 surfaces as indicated by the bead vortexing method and conductance measurement. *Journal of*

599 *Food Protection*, 68, 2149–2154.

600 Gurol, C., Ekinci, F., Aslan, N., & Korachi, M. (2012). Low temperature plasma for

601 decontamination of *E. coli* in milk. *International Journal of Food Microbiology*, *157*, 1-5.

602 Han, L., Patil, S., Keener, K. M., Cullen, P., & Bourke, P. (2014). Bacterial Inactivation by

603 High Voltage Atmospheric Cold Plasma: Influence of Process Parameters and Effects on Cell

604 Leakage and DNA. *Journal of Applied Microbiology*, 116, 784-794.

605 Hurst, A. (1977). Bacterial injury: A review. *Canadian Journal of Microbiology*, *23*, 935–

606   944.

607   Kayes, M. M., Critzer, F. J., Kelly-Wintenberg, K., Roth, J. R., Montie, T. C., & Golden,

608   D.A. (2007). Inactivation of Foodborne Pathogens Using a One Atmosphere Uniform Glow

609   Discharge Plasma. *Foodborne pathogens and disease*, 4, 50-59.

610   Kim, B., Hyejeong, Y., Jung, S., Jung, Y., Jung, H., Choe, W., & Jo, C. (2011). Effect of

611   atmospheric pressure plasma on inactivation of pathogens inoculated onto bacon using two

612   different gas compositions. *Food Microbiology*, *28*, 9-13.

613   Laroussi, M., & Leipold, F. (2004). Evaluation of the roles of reactive species, heat, and UV

614   radiation in the inactivation of bacterial cells by air plasmas at atmospheric pressure.

615   *International Journal of Mass Spectrometry*, 233, 81–86.

616   Laroussi, M. (2009). Low-Temperature Plasmas for Medicine? *IEEE Transactions on plasma*

617   *science*, 37, 714-725.

618   Lee, K., Paek, K., Ju, W., & Lee, Y. (2006). Sterilization of bacteria, yeast, and bacterial

619   endospores by atmospheric-pressure cold plasma using helium and oxygen. *Journal of*

620   *Microbiology*,  44, 269–275.

621   Lerouge, S., Wertheimer, M. R., & Yahia, L' H. (2001). Plasma Sterilization: A Review of

622   Parameters, Mechanisms, and Limitations. *Plasmas and Polymers*, 6, 175-188.

623   Leyer, G. J., & Johnson, E. A. (1993). Acid adaptation induces cross-protection against

624   environmental    stresses    in    *Salmonella*    Typhimurium.    *Applied    and    Environmental*

625   *Microbiology*, *59*, 1842-1847.

626   Li, J., Sakai, N., Watanabe, M., Hotta, E., & Wachi, M. (2013). Study on plasma agent effect

627   of a direct-current atmospheric pressure oxygen-plasma jet on inactivation of *E. coli* using

628    bacterial mutants. *IEEE Transactions on Plasma Science*, *41*, 935-941.

629    Lou, Y., & Yousef, A. E. (1997). Adaptation to sublethal environmental stresses protects

630    *Listeria monocytogenes* against lethal preservation factors. *Applied and Environmental*

631    *Microbiology*, *63*, 1252–1255.

632    Malakar, P. K, Brocklehurst, T. F., Mackie, A. R., Wilson, P. D. G, Zwietering, M. H., &

633    van't Riet, K. (2000). Microgradients in bacterial colonies: use of fluorescence ratio imaging,

634    a non-invasive technique. *International Journal of Food Microbiology*, *56*, 71–80.

635    Moisan M., Barbeau J., Moreau S., Pelletier J., Tabrizian M., & Yahia L. (2001). Low-

636    temperature sterilization using gas plasmas: a review of the experiments and an analysis of the

637    inactivation mechanisms. *International Journal of Pharmaceutics*, *226*, 1-21.

638    Moisan, M., Barbeau, J., Crevier, M.-C., Pelletier, J., Philip, N., & Saoudi, B. (2002). Plasma

639    sterilization. Methods and mechanisms. *Pure Applied Chemistry*, 74, 349–358.

640    Murakami, T., Niemi, K., Gans, T., O'Connell, D., & Graham, W. G. (2014). Afterglow

641    chemistry of atmospheric-pressure helium–oxygen plasmas with humid air impurity. *Plasma*

642    *Sources Science and Technology* 23, 025005 (12pp).

643    Niemira, B. A. (2012). Cold Plasma Decontamination of Foods. *Annual Review of Food*

644    *Science and Technology* 3, 125–142.

645    Noriega, E., Velliou, E., Van Derlinden, E., Mertens, L., & Van Impe, J. F. M. (2013). Effect

646    of cell immobilization on heat-induced sublethal injury of *Escherichia coli*, *Salmonella*

647    Typhimurium and *Listeria innocua*. *Food Microbiology*, *36*, 355-364.

648    Noriega, E., Velliou, E. G., Van Derlinden, E., Mertens, L., & Van Impe, J. F. M. (2014).

649    Role of growth morphology in the formulation of NaCl-based selective media for injury

650 detection of *Escherichia coli*, *Salmonella* Typhimurium and *Listeria innocua*. *Food Research*

651 *International*, 64, 402-411.

652 O'Byrne C. P., & Booth, I. R. (2002). Osmoregulation and its importance to foodborne

653 microorganisms. *International Journal of Food Microbiology*, *74*, 203-216.

654 Oehmigen, K., Hahnel, M., Brandenburg, R., Wilke, Ch., Weltmann, K.-D., & von Woedtke,

655 Th. (2010). The role of acidification for antimicrobial activity of atmospheric pressure plasma

656 in liquids. *Plasma Processes and Polymers*, *7*, 250-257.

657 Perni, S., Shama, G., Hobman, J. L., Lund, P. A., Kershaw, C. J., Hidalgo-Arroyo, G. A.,

658 Penn, C. W., Deng, X. T., Walsh, J. L., & Kong, M. G. (2007). Probing bactericidal

659 mechanisms induced by cold atmospheric plasmas with *Escherichia coli* mutants. *Applied*

660 *Physics Letters*, *90*:073902.

661 Rees, C. E. D., Dodd, C. E. R., Gibson, P. T., Booth, I. R., & Stewart, G. S. A. B. (1995). The

662 significance of bacteria in stationary phase to food microbiology. *International Journal of*

663 *Food Microbiology*, *28*, 263–275.

664 Rowan, N. J., Espie, S., Harrower, J., Anderson, J. G, Marsili, L., & MacGregor, S. J.

665 (2007a). Pulsed-Plasma Gas-Discharge Inactivation of Microbial Pathogens in Chilled Poultry

666 Wash Water. *Journal of Food Protection*, 70, 2805–2810.

667 Rowan, N. J., Espie, S., Harrower, J., Farell, H., Marsili, L., Anderson, J. G., & MacGregor,

668 S. J. (2007b). Evidence of lethal and sublethal injury in food-borne bacterial pathogens

669 exposed to high-intensity pulsed-plasma gas discharges. *Letters in Applied Microbiology*, 46,

670 80-86.

671    Selcuk, M., Oksuz, L., & Basaran, P. (2008). Decontamination of grains and legumes infected

672    with *Aspergillus* spp. and *Penicillum* spp. by cold plasma treatment. *Bioresource Technology*,

673    *99*, 5104-5109.

674    Smet, C., Noriega, E., Van Mierlo, J., Valdramidis, V. P., & Van Impe, J. F. (2015). Influence

675    of the growth morphology on the behavior of *Salmonella* Typhimurium and *Listeria*

676    *monocytogenes* under osmotic stress. *Food Research International*, *77*, 515-526.

677    Smet, C., Noriega, E., Matsoukas, I., Rosier, F., Walsh, J. L., Valdramidis, V. P., & Van

678    Impe, J. F. (2016). The role of the food structure on the inactivation efficacy of cold

679    atmospheric plasma. *International Journal of Food Microbiology*, submitted.

680    Vleugels, M., Shama, G., Deng, X. T., Greenacre, E. J., Brocklehurst, T. F., & Kong, M. G.

681    (2005). Atmospheric plasma inactivation of biofilm-forming bacteria for food safety control.

682    *Institute of Electrical and Electronics Engineers Transactions on Plasma Science*, 33, 824–

683    828.

684    Wesche, A., Gurtler, J. B., Marks, B. P., & Ryser, E. T. (2009). Stress, sublethal injury,

685    resuscitation, and virulence of bacterial foodborne pathogens. *Journal of Food Protection*, *72*,

686    1121-1138.

687    Wimpenny, J. W. T., & Coombs, J. P. (1983). Penetration of oxygen into bacterial colonies.

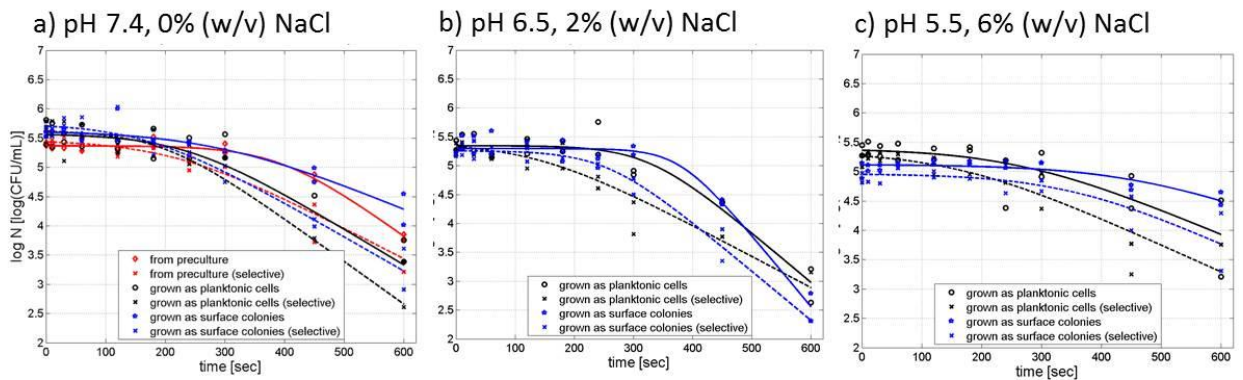688    *Journal of General Microbiology*, *129*, 1239–1242.
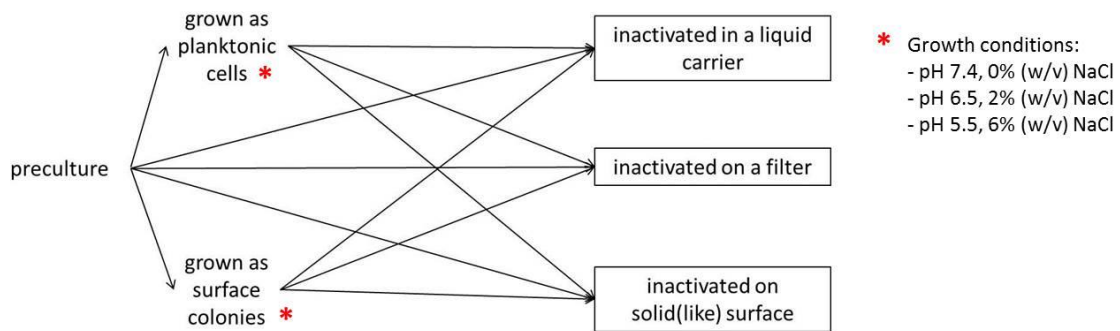
689

690    Figure 1: Different combinations tested at each experimental condition. Cells were inactivated

691    in a liquid carrier (a, b, c), on a solid(like) surface (d, e, f) and on a filter (g, h, i). Prior to

692    CAP treatment, cells were grown at  pH 7.4, 0% (w/v) NaCl (a, d, g), pH 6.5, 2% (w/v) NaCl

693    (b, e, h)  or pH 5.5, 6% (w/v) NaCl (c, f, i), and either planktonically or as surface colonies.

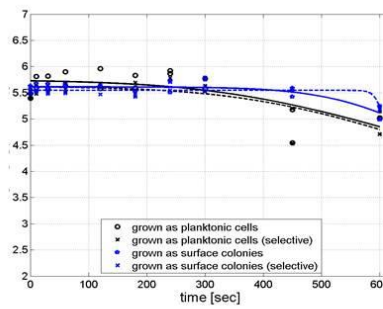694    For pH 7.4, 0% (w/v) NaCl (a, d, g), as a control, cells from the preculture were directly

695    treated. (b) Examples for the different combinations.

a)

b)

c)
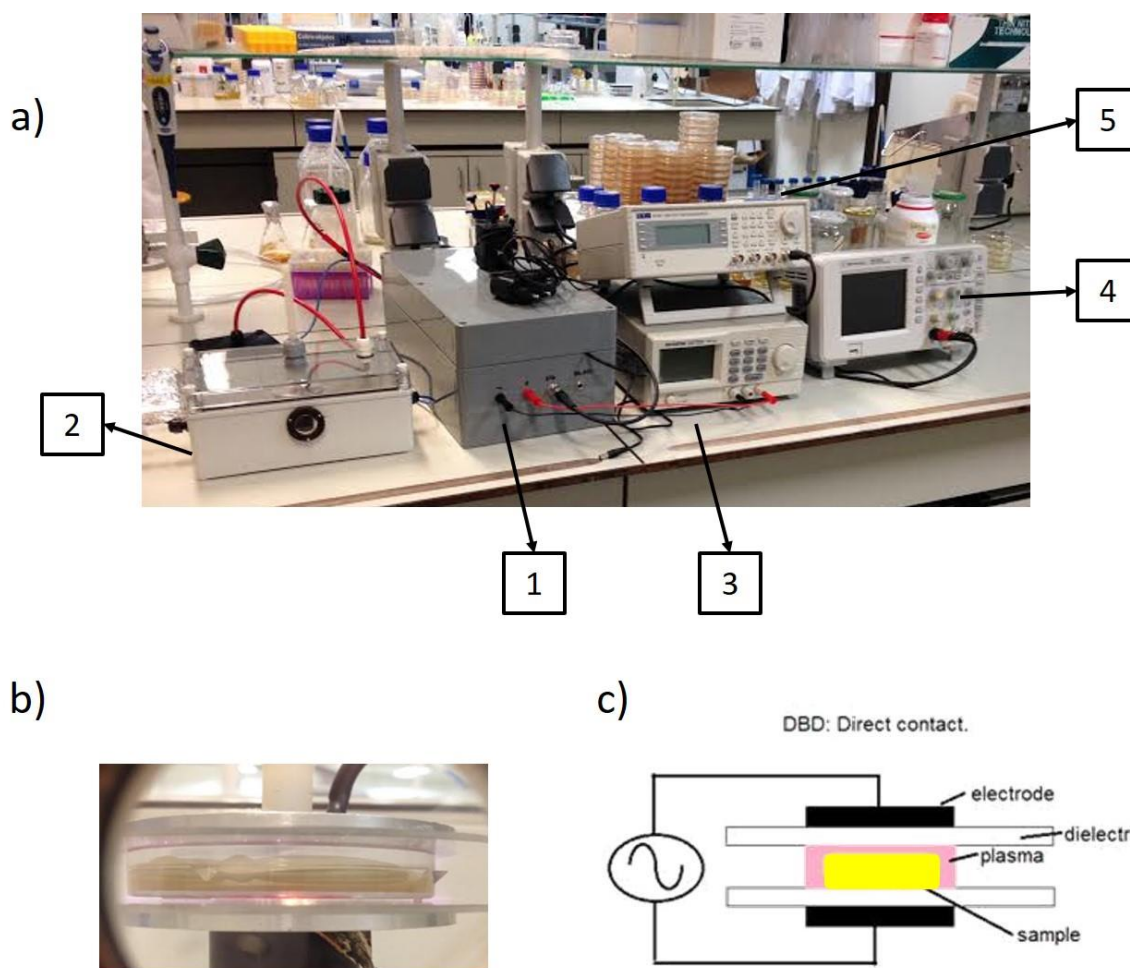
696

697 Figure 2: (a) the CAP set up: (1) plasma power source, (2) Dielectric Barrier Discharge

698 reactor (22.5 cm x 13.5 cm x 10 cm), (3) DC power supply, (4) oscilloscope and (5) function

699 generator. (b) DBD electrode inside reactor (electrode: diameter 5.5 cm, dielectric: 7.5 cm)

700 petri dish containing sample: petri dish with diameter 5 cm). (c) Schematic representation

701 DBD electrode with sample.
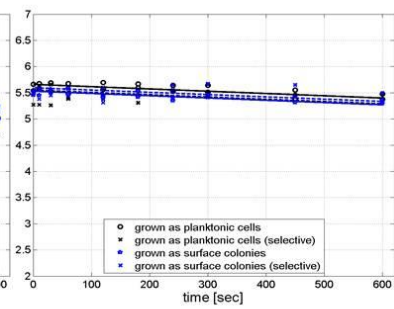
**Inactivated in a liquid carrier**

a) pH 7.4, 0% (w/v) NaCl
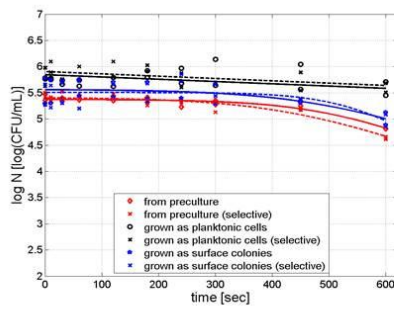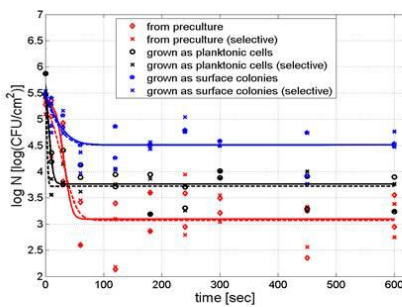
b) pH 6.5, 2% (w/v) NaCl
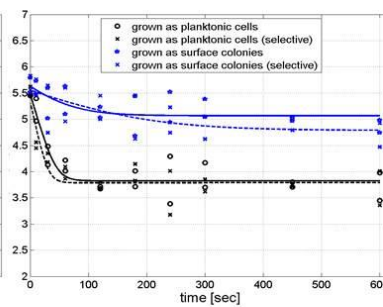
c) pH 5.5, 6% (w/v) NaCl

**Inactivated on a solid(like) surface**

d) pH 7.4, 0% (w/v) NaCl

e) pH 6.5, 2% (w/v) NaCl

f) pH 5.5, 6% (w/v) NaCl
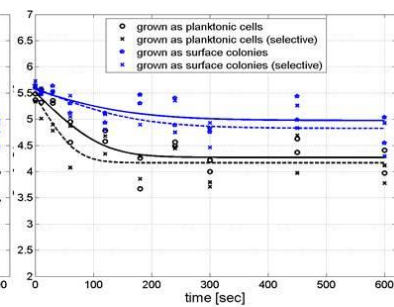
**Inactivated on a filter**

g) pH 7.4, 0% (w/v) NaCl
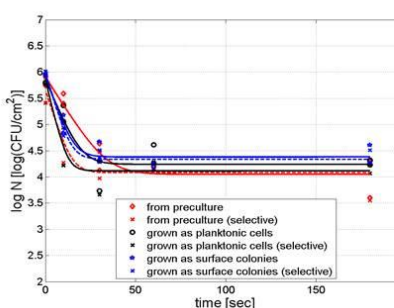
h) pH 6.5, 2% (w/v) NaCl

i) pH 5.5, 6% (w/v) NaCl

Figure 3: Survival curves of stationary phase *S.* Typhimurium after exposure to CAP. Cells were inactivated in a liquid carrier (a, b, c), on a solid(like) surface (d, e, f) and on a filter (g, h, i). Prior to CAP treatment, cells were grown at pH 7.4, 0% (w/v) NaCl (a, d, g), pH 6.5, 2% (w/v) NaCl (b, e, h) or pH 5.5, 6% (w/v) NaCl (c, f, i), and either planktonically or as surface colonies. For pH 7.4, 0% (w/v) NaCl (a, d, g), as a control, cells from the preculture were

708    directly treated. Experimental data (symbols) and global fit (line) of the Geeraerd et al. (2000)

709    model: total viable population (o, solid line) and uninjured viable population (x, dashed line).

Figure 4: Survival curves of stationary phase *L. monocytogenes* after exposure to CAP. Cells were inactivated in a liquid carrier (a, b, c), on a solid(like) surface (d, e, f) and on a filter (g, h, i). Prior to CAP treatment, cells were grown at  pH 7.4, 0% (w/v) NaCl (a, d, g), pH 6.5, 2% (w/v) NaCl (b, e, h)  or pH 5.5, 6% (w/v) NaCl (c, f, i), and either planktonically or as surface colonies. For pH 7.4, 0% (w/v) NaCl (a, d, g), as a control, cells from the preculture were directly treated.  Experimental data (symbols) and global fit (line) of the Geeraerd et al.

717    (2000) model: total viable population (o, solid line) and uninjured viable population (x,

718    dashed line).
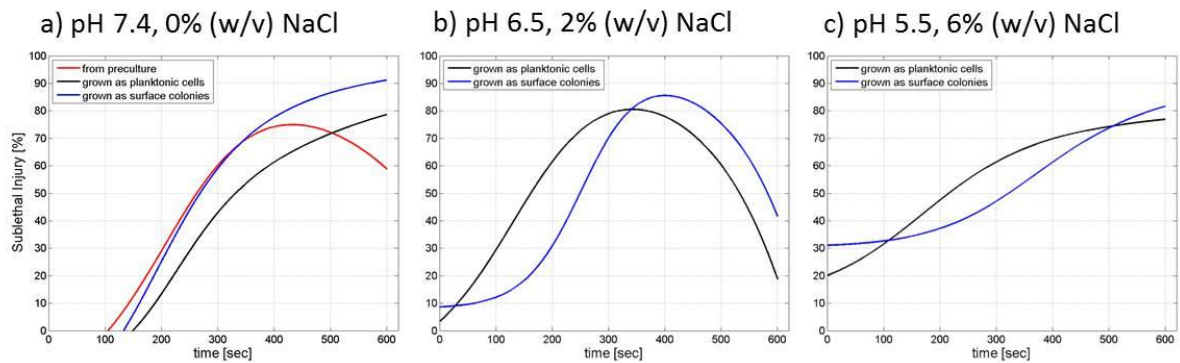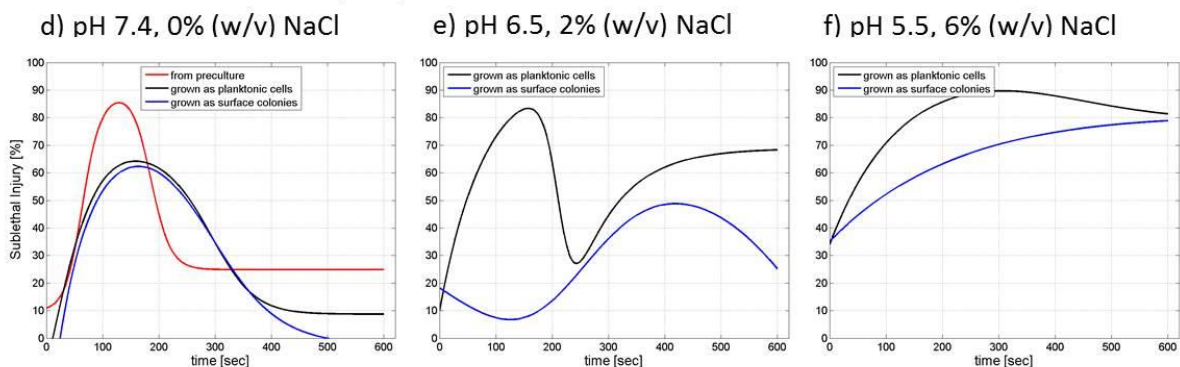
Figure 5: Evolution with time of the sublethal injury (%) of *S.* Typhimurium towards the exposure time to CAP. Cells were inactivated as a liquid (a, b, c), on a solid(like) surface (d, e, f) and on a filter (g, h, i). Prior to CAP treatment, cells were grown at pH 7.4, 0% (w/v) NaCl (a, d, g), pH 6.5, 2% (w/v) NaCl (b, e, h) or pH 5.5, 6% (w/v) NaCl (c, f, i), and either planktonically or as surface colonies. For pH 7.4, 0% (w/v) NaCl (a, d, g), as a control, cells from the preculture were directly treated.

Figure 6: Evolution with time of the sublethal injury (%) of *L. monocytogenes* towards the exposure time to CAP. Cells were inactivated as a liquid (a, b, c), on a solid(like) surface (d, e, f) and on a filter (g, h, i). Prior to CAP treatment, cells were grown at pH 7.4, 0% (w/v) NaCl (a, d, g), pH 6.5, 2% (w/v) NaCl (b, e, h) or pH 5.5, 6% (w/v) NaCl (c, f, i), and either planktonically or as surface colonies. For pH 7.4, 0% (w/v) NaCl (a, d, g), as a control, cells from the preculture were directly treated.

733 Table 1. Inactivation parameters of the Geeraerd et al. (2000) model for *S.* Typhimurium after exposure to CAP. Cells were inactivated on a

734 liquid carrier, on a solid(like) surface and on a filter. Prior to CAP treatment, cells were grown at pH 7.4, 0% (w/v) NaCl, pH 6.5, 2% (w/v) NaCl

735 or pH 5.5, 6% (w/v) NaCl, and either planktonically, as surface colonies, or as the direct preculture (only for pH 7.4, 0% (w/v) NaCl).

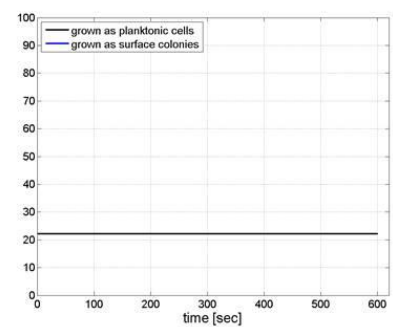| Inactivation support system | Growth conditions | | | Growth morphology | Population | Inactivation parameters | | | | RMSE | $_1$log reduction$_2$$^3$ |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | pH (-) | NaCl (% (w/v)) | Reference | | | $_1$log N$_0$ (log (CFU/mL))$_2$$^3$ / $_1$log N$_0$ (log (CFU/cm$^2$))$_2$$^3$ | $_1$t$_l$ (s)$_2$$^3$ | $_1$k$_{max}$ (1/s)$_2$$^3$ | $_1$log N$_{res}$ (log(CFU/mL))$_2$$^3$ / $_1$log N$_{res}$ (log (CFU/cm$^2$))$_2$$^3$ | | |
| *liquid* | 7.4 | 0 | a | Preculture | Total | 5.4 ± 0.0$_A$ | (b)392.2 ± 21.1$_B$ | (a)0.017 ± 0.002$_B$ | - | 0.1122 | ≈ (b)1.6 ± 0.0$_B$ |
| | | | | | Uninjured | 5.4 ± 0.1$_A$ | (b)220.6 ± 41.5$_A$ | (a)0.012 ± 0.002$_A$ | - | 0.1996 | ≈ (a)2.0 ± 0.1$_A$ |
| | | | | Planktonic cells | Total | 5.6 ± 0.1$_B$$^b$ | (b)238.3 ± 74.3$_A$$^a$ | (a)0.014 ± 0.003$_{AB}$$^a$ | - | 0.4204 | ≈ (a)2.3 ± 0.1$_C$$^b$ |
| | | | | | Uninjured | 5.6 ± 0.2$_{AB}$$^b$ | (b)196.8 ± 58.7$_A$$^a$ | (a)0.017 ± 0.003$_B$$^b$ | - | 0.4567 | ≈ (b)2.9 ± 0.2$_C$$^c$ |
| | | | | Surface colonies | Total | 5.6 ± 0.1$_B$$^c$ | (b)296.8 ± 47.5$_{AB}$$^a$ | (a)0.010 ± 0.002$_A$$^a$ | - | 0.1567 | ≈ (a)1.3 ± 0.1$_A$$^b$ |
| | | | | | Uninjured | 5.7 ± 0.1$_B$$^c$ | 177.4 ± 34.9$_A$$^a$ | (a)0.014 ± 0.001$_{AB}$$^a$ | - | 0.2061 | ≈ (c)2.5 ± 0.1$_B$$^b$ |
| | 6.5 | 2 | b | Planktonic cells | Total | 5.3 ± 0.1$_A$$^a$ | 325.4 ± 31.6$_A$$^a$ | (a)0.020 ± 0.003$_A$$^b$ | - | 0.2167 | ≈ (c)2.3 ± 0.1$_A$$^b$ |
| | | | | | Uninjured | 5.3 ± 0.1$_A$$^a$ | 150.2 ± 60.0$_A$$^a$ | (a)0.013 ± 0.002$_A$$^{ab}$ | - | 0.3217 | ≈ (b)2.4 ± 0.1$_A$$^b$ |
| | | | | Surface colonies | Total | 5.3 ± 0.0$_A$$^b$ | (b)378.4 ± 18.7$_A$$^b$ | (a)0.029 ± 0.003$_B$$^b$ | - | 0.1833 | ≈ (c)2.7 ± 0.0$_B$$^c$ |
| | | | | | Uninjured | 5.3 ± 0.0$_A$$^b$ | 255.5 ± 17.3$_B$$^b$ | (a)0.020 ± 0.001$_B$$^b$ | - | 0.1443 | ≈ (c)3.0 ± 0.0$_B$$^c$ |
| | 5.5 | 6 | c | Planktonic cells | Total | 5.4 ± 0.1$_B$$^a$ | 271.8 ± 96.4$_A$$^a$ | (a)0.010 ± 0.003$_A$$^a$ | - | 0.3326 | ≈ (a)1.5 ± 0.1$_B$$^a$ |
| | | | | | Uninjured | 5.3 ± 0.1$_B$$^a$ | 174.0 ± 64.0$_A$$^a$ | (a)0.011 ± 0.002$_A$$^a$ | - | 0.2697 | ≈ (a)2.0 ± 0.1$_B$$^a$ |
| | | | | Surface colonies | Total | 5.0 ± 0.0$_A$$^a$ | 473.7 ± 41.5$_B$$^c$ | (a)0.009 ± 0.003$_A$$^a$ | - | 0.1079 | ≈ (a)0.5 ± 0.0$_A$$^a$ |
| | | | | | Uninjured | 5.0 ± 0.1$_A$$^a$ | 351.1 ± 70.1$_B$$^b$ | (b)0.011 ± 0.003$_A$$^a$ | - | 0.2391 | ≈ (b)1.2 ± 0.1$_A$$^a$ |
| *solid(like) surface* | 7.4 | 0 | d | Preculture | Total | 5.5 ± 0.1$_A$ | (a)88.9 ± 21.4$_A$ | (b)0.057 ± 0.019$_B$ | 3.1 ± 0.1$_A$ | 0.3386 | (c)2.4 ± 0.1$_B$ |
| | | | | | Uninjured | 5.4 ± 0.1$_A$ | (a)61.8 ± 12.9$_A$ | (b)0.065 ± 0.014$_B$ | 3.0 ± 0.1$_A$ | 0.1970 | (b)2.4 ± 0.1$_B$ |
| | | | | Planktonic cells | Total | 5.6 ± 0.2$_A$$^b$ | (a)95.8 ± 54.3$_A$ | (a)0.026 ± 0.010$_A$$^a$ | 3.3 ± 0.2$_A$$^a$ | 0.4455 | (a)2.3 ± 0.3$_B$$^c$ |
| | | | | | Uninjured | 5.7 ± 0.2$_{AB}$$^b$ | (a)33.0 ± 57.6$_A$ | (a)0.023 ± 0.008$_A$$^a$ | 3.3 ± 0.2$_A$$^{ab}$ | 0.3842 | (a)2.4 ± 0.3$_B$$^b$ |
| | | | | Surface colonies | Total | 5.6 ± 0.2$_A$$^b$ | (a)117.1 ± 113.5$_A$$^a$ | (ab)0.017 ± 0.015$_A$$^a$ | 4.4 ± 0.3$_B$ | 0.4549 | (a)1.2 ± 0.4$_A$$^b$ |
| | | | | | Uninjured | 5.8 ± 0.2$_B$$^c$ | - | (a)0.017 ± 0.005$_A$$^b$ | 4.4 ± 0.2$_B$ | 0.3666 | (a)1.4 ± 0.3$_A$$^b$ |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 6.5 | 2 | e | Planktonic cells | Total | $5.2 \pm 0.1_A{}^a$ | $180.2 \pm 14.8_A$ | $(a)0.073 \pm 0.063_A{}^a$ | $4.1 \pm 0.1^b$ | 0.2571 | $(a)1.1 \pm 0.1_A{}^a$ |
| | | | | | Uninjured | $5.1 \pm 0.1_A{}^a$ | - | $(a)0.013 \pm 0.003_A{}^a$ | $3.6 \pm 0.2_A{}^b$ | 0.3446 | $(a)1.5 \pm 0.2_B{}^a$ |
| | | | | Surface colonies | Total | $5.6 \pm 0.1_B{}^b$ | $(ab)140.7 \pm 199.4_A{}^a$ | $(a)0.004 \pm 0.002_A{}^a$ | - | 0.1514 | $\approx (a)0.9 \pm 0.1_A{}^{ab}$ |
| | | | | | Uninjured | $5.5 \pm 0.1_B{}^b$ | $214.0 \pm 40.4$ | $(a)0.014 \pm 0.007_A{}^b$ | $4.6 \pm 0.1_B$ | 0.1377 | $(a)0.9 \pm 0.1_A{}^a$ |
| | 5.5 | 6 | f | Planktonic cells | Total | $5.4 \pm 0.1_B{}^{ab}$ | - | $(a)0.009 \pm 0.002_A{}^a$ | $3.8 \pm 0.2^b$ | 0.2830 | $(a)1.6 \pm 0.2_B{}^b$ |
| | | | | | Uninjured | $5.2 \pm 0.1_B{}^a$ | - | $(b)0.018 \pm 0.003_B{}^a$ | $3.1 \pm 0.2^a$ | 0.3246 | $(a)2.1 \pm 0.2_B{}^b$ |
| | | | | Surface colonies | Total | $5.1 \pm 0.1_A{}^a$ | $350.3 \pm 189.6^a$ | $(a)0.005 \pm 0.003_A{}^a$ | - | 0.1919 | $\approx (a)0.6 \pm 0.1_A{}^a$ |
| | | | | | Uninjured | $4.9 \pm 0.1_A{}^a$ | - | $(a)0.004 \pm 0.000_A{}^a$ | - | 0.3223 | $\approx (b)1.1 \pm 0.1_A{}^{ab}$ |
| *filter* | 7.4 | 0 | g | Preculture | Total | $5.5 \pm 0.2_A$ | $(a)2.2 \pm 163.3_A$ | $(a)0.014 \pm 0.011_A$ | - | 0.2917 | $\approx (a)1.0 \pm 0.2_A$ |
| | | | | | Uninjured | $5.5 \pm 0.1_A$ | $(a)13.6 \pm 32.6_A$ | $(a)0.027 \pm 0.005_A$ | - | 0.2149 | $\approx (a)2.0 \pm 0.1_A$ |
| | | | | Planktonic cells | Total | $5.7 \pm 0.1_A{}^b$ | $(a)12.4 \pm 15.1_A$ | $(b)0.057 \pm 0.017_B{}^b$ | $3.5 \pm 0.1$ | 0.1482 | $(a)2.2 \pm 0.1_C{}^b$ |
| | | | | | Uninjured | $5.6 \pm 0.1_A{}^b$ | $(a)10.1 \pm 7.6_A$ | $(b)0.097 \pm 0.019_B{}^{ab}$ | $3.4 \pm 0.1$ | 0.1699 | $(a)2.2 \pm 0.1_A{}^a$ |
| | | | | Surface colonies | Total | $5.5 \pm 0.1_A{}^b$ | $(a)89.0 \pm 47.6_A$ | $(b)0.045 \pm 0.022_{AB}{}^a$ | - | 0.2613 | $\approx (b)1.8 \pm 0.1_B{}^c$ |
| | | | | | Uninjured | $5.5 \pm 0.2_A{}^b$ | $20.1 \pm 52.6_A$ | $(b)0.029 \pm 0.009_A{}^b$ | - | 0.3880 | $\approx (b)2.0 \pm 0.2_A{}^c$ |
| | 6.5 | 2 | h | Planktonic cells | Total | $5.5 \pm 0.2_A{}^{ab}$ | - | $(a)0.021 \pm 0.004_A{}^a$ | - | 0.3967 | $\approx (b)1.7 \pm 0.2_A{}^a$ |
| | | | | | Uninjured | $5.4 \pm 0.2_A{}^b$ | $25.4 \pm 15.5$ | $(b)0.118 \pm 0.063_B{}^b$ | $3.5 \pm 0.3$ | 0.4374 | $(ab)1.9 \pm 0.4_A{}^a$ |
| | | | | Surface colonies | Total | $5.8 \pm 0.0_A{}^c$ | $(a)115.1 \pm 79.7$ | $(a)0.049 \pm 0.059_A{}^a$ | - | 0.2019 | $\approx (b)1.4 \pm 0.0_A{}^b$ |
| | | | | | Uninjured | $5.8 \pm 0.2_A{}^b$ | - | $(a)0.014 \pm 0.005_A{}^a$ | - | 0.4058 | $\approx (b)1.2 \pm 0.2_A{}^b$ |
| | 5.5 | 6 | i | Planktonic cells | Total | $5.3 \pm 0.1_A{}^a$ | $25.1 \pm 49.0$ | $(b)0.022 \pm 0.007_B{}^a$ | - | 0.2178 | $\approx (a)1.5 \pm 0.1_B{}^a$ |
| | | | | | Uninjured | $5.0 \pm 0.1_A{}^a$ | - | $(c)0.028 \pm 0.003_B{}^a$ | - | 0.2775 | $\approx (a)2.2 \pm 0.1_B{}^a$ |
| | | | | Surface colonies | Total | $5.2 \pm 0.1_A{}^a$ | - | $(a)0.006 \pm 0.003_A{}^a$ | - | 0.2667 | $\approx (a)0.5 \pm 0.1_A{}^a$ |
| | | | | | Uninjured | $4.7 \pm 0.2_A{}^a$ | - | $(ab)0.007 \pm 0.005_A{}^a$ | - | 0.4573 | $\approx (a)0.5 \pm 0.2_A{}^a$ |

736
737

[1] For each experimental condition, growth morphology and population type, parameters of the Geeraerd model bearing different subscripts (no lowercase letters in common) are significantly different ($P \leq 0.05$)

[2] For each inactivation support, experimental condition and population type, parameters of the Geeraerd model bearing different subscripts (no uppercase letters in common) are significantly different ($P \leq 0.05$)

[3] For each inactivation support, growth morphology and population type, parameters of the Geeraerd model bearing different superscripts (no lowercase letters in common) are significantly different ($P \leq 0.05$)

738 Table 2. Inactivation parameters of the Geeraerd et al. (2002) model for *L. monocytogenes* after exposure to CAP. Cells were inactivated on a

739 liquid carrier, on a solid(like) surface and on a filter. Prior to CAP treatment, cells were grown at pH 7.4, 0% (w/v) NaCl, pH 6.5, 2% (w/v) NaCl

740 or pH 5.5, 6% (w/v) NaCl, and either planktonically, as surface colonies, or as the direct preculture (only for pH 7.4, 0% (w/v) NaCl).

| Inactivation support system | Growth conditions | | | Growth morphology | Population | Kinetic parameters | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | pH (-) | NaCl (% (w/v)) | Reference | | | $_1\log N_0$ $(\log(CFU/mL))_2^3$ / $_1\log N_0$ $(\log(CFU/cm^2))_2^3$ | $_1 t_l (s)_2^3$ | $_1 k_{max} (1/s)_2^3$ | $_1\log N_{res}$ $(\log(CFU/mL))_2^3$ / $_1\log N_{res}$ $(\log(CFU/cm^2))_2^3$ | RMSE | $_1\log reduction_2^3$ |
| *Liquid* | 7.4 | 0 | a | Preculture | Total | $5.4 \pm 0.0_A$ | $511.9 \pm 18.0$ | $_{(a)}0.011 \pm 0.002_B$ | - | 0.0557 | $\approx_{(a)}0.6 \pm 0.0_B$ |
| | | | | | Uninjured | $5.4 \pm 0.0_A$ | $438.0 \pm 30.5$ | $_{(a)}0.010 \pm 0.002_A$ | - | 0.0822 | $\approx_{(a)}0.8 \pm 0.0_C$ |
| | | | | Planktonic cells | Total | $5.8 \pm 0.1_C{}^a$ | - | $_{(a)}0.001 \pm 0.000_A{}^a$ | - | 0.1818 | $\approx_{(a)}0.2 \pm 0.1_A{}^a$ |
| | | | | | Uninjured | $5.9 \pm 0.1_B{}^b$ | - | $_{(a)}0.001 \pm 0.000_A{}^a$ | - | 0.1633 | $\approx_{(a)}0.3 \pm 0.1_A{}^a$ |
| | | | | Surface colonies | Total | $5.6 \pm 0.1_B{}^a$ | $493.0 \pm 70.0$ | $_{(a)}0.009 \pm 0.005_B{}^{ab}$ | - | 0.1840 | $\approx_{(a)}0.6 \pm 0.1_B{}^b$ |
| | | | | | Uninjured | $5.5 \pm 0.1_A{}^a$ | $540.0 \pm 53.5$ | $_{(a)}0.015 \pm 0.012_A{}^b$ | - | 0.2031 | $\approx_{(a)}0.5 \pm 0.1_B{}^b$ |
| | 6.5 | 2 | b | Planktonic cells | Total | $5.7 \pm 0.1_A{}^a$ | $369.3 \pm 108.7_A$ | $_{(a)}0.008 \pm 0.004_A{}^b$ | - | 0.2638 | $\approx_{(a)}0.9 \pm 0.1_B{}^b$ |
| | | | | | Uninjured | $5.6 \pm 0.1_A{}^a$ | $407.6 \pm 91.4_A$ | $_{(a)}0.009 \pm 0.004_A{}^b$ | - | 0.2403 | $\approx_{(a)}0.8 \pm 0.1_B{}^b$ |
| | | | | Surface colonies | Total | $5.6 \pm 0.0_A{}^a$ | $546.3 \pm 24.4_A$ | $_{(a)}0.014 \pm 0.005_A{}^b$ | - | 0.0919 | $\approx_{(a)}0.5 \pm 0.0_A{}^b$ |
| | | | | | Uninjured | $5.5 \pm 0.0_A{}^a$ | $598.8 \pm 2.2_B$ | $_{(b)}0.123 \pm 0.001_B{}^c$ | - | 0.0872 | $\approx_{(a)}0.4 \pm 0.0_A{}^{ab}$ |
| | 5.5 | 6 | c | Planktonic cells | Total | $5.7 \pm 0.0_B{}^a$ | - | $_{(a)}0.001 \pm 0.000_A{}^a$ | - | 0.0815 | $\approx_{(a)}0.3 \pm 0.0_B{}^a$ |
| | | | | | Uninjured | $5.6 \pm 0.1_A{}^a$ | - | $_{(a)}0.001 \pm 0.000_A{}^a$ | - | 0.1691 | $\approx_{(a)}0.3 \pm 0.1_A{}^a$ |
| | | | | Surface colonies | Total | $5.5 \pm 0.0_A{}^a$ | - | $_{(a)}0.001 \pm 0.000_A{}^a$ | - | 0.0581 | $\approx_{(a)}0.2 \pm 0.0_A{}^a$ |
| | | | | | Uninjured | $5.6 \pm 0.0_A{}^a$ | - | $_{(a)}0.001 \pm 0.000_A{}^a$ | - | 0.1382 | $\approx_{(a)}0.3 \pm 0.0_A{}^a$ |
| *solid(like) surface* | 7.4 | 0 | d | Preculture | Total | $5.3 \pm 0.3_A$ | $20.5 \pm 27.6$ | $_{(a)}0.219 \pm 0.576_A$ | $3.1 \pm 0.1_A$ | 0.4751 | $_{(b)}2.2 \pm 0.3_B$ |
| | | | | | Uninjured | $5.2 \pm 0.3_A$ | $9.8 \pm 19.5$ | $_{(ab)}0.119 \pm 0.088_A$ | $3.1 \pm 0.1_A$ | 0.5108 | $_{(c)}2.1 \pm 0.3_B$ |
| | | | | Planktonic cells | Total | $5.7 \pm 0.2_A{}^a$ | - | $_{(c)}0.355 \pm 0.096_A{}^b$ | $3.8 \pm 0.1_B{}^a$ | 0.3335 | $_{(b)}1.9 \pm 0.2_B{}^b$ |
| | | | | | Uninjured | $5.7 \pm 0.2_B{}^b$ | - | $_{(a)}1.000 \pm 13.908_A{}^a$ | $3.7 \pm 0.1_B{}^a$ | 0.3103 | $_{(b)}2.0 \pm 0.2_B{}^b$ |
| | | | | Surface colonies | Total | $5.5 \pm 0.2_A{}^a$ | - | $_{(a)}0.070 \pm 0.031_A{}^b$ | $4.5 \pm 0.1_C{}^a$ | 0.2594 | $_{(b)}1.0 \pm 0.2_A{}^b$ |
| | | | | | Uninjured | $5.4 \pm 0.2_{AB}{}^a$ | - | $_{(a)}0.077 \pm 0.048_A{}^b$ | $4.5 \pm 0.1_C{}^a$ | 0.3503 | $_{(b)}0.9 \pm 0.2_A{}^a$ |

| | pH | n | | Growth morphology | Population | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 6.5 | 2 | e | Planktonic cells | Total | $5.5 \pm 0.2_A{}^a$ | - | $_{(b)}0.088 \pm 0.021_B{}^a$ | $3.8 \pm 0.1_A{}^a$ | 0.2568 | $_{(b)}1.7 \pm 0.2_B{}^b$ |
| | | | | | Uninjured | $5.3 \pm 0.2_A{}^a$ | - | $_{(b)}0.120 \pm 0.035_B{}^a$ | $3.8 \pm 0.1_A{}^a$ | 0.2820 | $_{(c)}1.5 \pm 0.2_B{}^a$ |
| | | | | Surface colonies | Total | $5.6 \pm 0.1_A{}^a$ | - | $_{(a)}0.023 \pm 0.017_A{}^a$ | $5.1 \pm 0.1_B{}^b$ | 0.2629 | $_{(a)}0.5 \pm 0.1_A{}^a$ |
| | | | | | Uninjured | $5.6 \pm 0.1_A{}^a$ | - | $_{(a)}0.010 \pm 0.006_A{}^a$ | $4.8 \pm 0.2_B{}^b$ | 0.3169 | $_{(b)}0.8 \pm 0.2_A{}^a$ |
| | 5.5 | 6 | f | Planktonic cells | Total | $5.5 \pm 0.1_A{}^a$ | - | $_{(a)}0.027 \pm 0.008_A{}^a$ | $4.3 \pm 0.1_B{}^b$ | 0.2646 | $_{(c)}1.2 \pm 0.1_B{}^a$ |
| | | | | | Uninjured | $5.5 \pm 0.2_A{}^{ab}$ | - | $_{(a)}0.046 \pm 0.018_B{}^a$ | $4.2 \pm 0.1_B{}^b$ | 0.3330 | $_{(c)}1.3 \pm 0.2_B{}^a$ |
| | | | | Surface colonies | Total | $5.6 \pm 0.1_A{}^a$ | - | $_{(a)}0.012 \pm 0.007_A{}^a$ | $5.0 \pm 0.1_B{}^b$ | 0.2442 | $_{(b)}0.6 \pm 0.1_A{}^a$ |
| | | | | | Uninjured | $5.6 \pm 0.1_A{}^a$ | - | $_{(a)}0.013 \pm 0.007_A{}^a$ | $4.8 \pm 0.1_B{}^b$ | 0.2807 | $_{(b)}0.8 \pm 0.1_A{}^a$ |
| *filter* | 7.4 | 0 | g | Preculture | Total | $5.9 \pm 0.2_A$ | - | $_{(a)}0.127 \pm 0.030_A$ | $4.0 \pm 0.1_A$ | 0.2721 | $_{(b)}1.9 \pm 0.2_B$ |
| | | | | | Uninjured | $5.6 \pm 0.3_A$ | - | $_{(b)}0.252 \pm 0.100_A$ | $4.0 \pm 0.1_A$ | 0.3586 | $_{(b)}1.6 \pm 0.3_A$ |
| | | | | Planktonic cells | Total | $5.9 \pm 0.2_A{}^b$ | - | $_{(b)}0.190 \pm 0.067_{AB}{}^a$ | $4.2 \pm 0.1_B{}^a$ | 0.2804 | $_{(b)}1.7 \pm 0.2_{AB}{}^b$ |
| | | | | | Uninjured | $5.8 \pm 0.2_A{}^b$ | - | $_{(a)}0.333 \pm 0.085_A{}^a$ | $4.1 \pm 0.1_A{}^a$ | 0.2820 | $_{(b)}1.7 \pm 0.2_A{}^b$ |
| | | | | Surface colonies | Total | $5.9 \pm 0.1_A{}^b$ | - | $_{(b)}0.235 \pm 0.051_B{}^a$ | $4.4 \pm 0.1_C{}^a$ | 0.1876 | $_{(c)}1.5 \pm 0.1_A{}^c$ |
| | | | | | Uninjured | $6.0 \pm 0.1_A{}^b$ | - | $_{(b)}0.265 \pm 0.042_A{}^a$ | $4.3 \pm 0.1_B{}^a$ | 0.1516 | $_{(c)}1.7 \pm 0.1_A{}^b$ |
| | 6.5 | 2 | h | Planktonic cells | Total | $5.4 \pm 0.2_A{}^a$ | - | $_{(b)}0.075 \pm 0.035_A{}^a$ | $4.4 \pm 0.2_A{}^a$ | 0.2764 | $_{(a)}1.1 \pm 0.3_A{}^a$ |
| | | | | | Uninjured | $5.4 \pm 0.1_A{}^a$ | - | $_{(b)}0.100 \pm 0.037_A{}^a$ | $4.3 \pm 0.1_A{}^b$ | 0.2167 | $_{(b)}1.1 \pm 0.1_B{}^a$ |
| | | | | Surface colonies | Total | $5.3 \pm 0.1_A{}^a$ | $53.0 \pm 31.3$ | $_{(a)}0.158 \pm 0.666_A{}^a$ | $4.5 \pm 0.2_A{}^a$ | 0.2123 | $_{(b)}0.8 \pm 0.2_A{}^b$ |
| | | | | | Uninjured | $5.2 \pm 0.1_A{}^a$ | $50.4 \pm 23.0$ | $_{(ab)}0.060 \pm 0.093_A{}^a$ | $4.4 \pm 0.1_A{}^a$ | 0.1915 | $_{(b)}0.8 \pm 0.1_A{}^a$ |
| | 5.5 | 6 | i | Planktonic cells | Total | $5.3 \pm 0.1_A{}^a$ | $29.7 \pm 5.6$ | $_{(a)}0.205 \pm 0.888_A{}^a$ | $4.3 \pm 0.1_A{}^a$ | 0.2097 | $_{(b)}1.0 \pm 0.1_A{}^a$ |
| | | | | | Uninjured | $5.4 \pm 0.1_A{}^a$ | $20.2 \pm 12.9$ | $_{(a)}0.203 \pm 0.240_A{}^a$ | $4.2 \pm 0.1_A{}^{ab}$ | 0.1866 | $_{(b)}1.2 \pm 0.1_B{}^a$ |
| | | | | Surface colonies | Total | $5.5 \pm 0.2_A{}^a$ | - | $_{(a)}0.113 \pm 0.164_A{}^a$ | $5.0 \pm 0.2_B{}^b$ | 0.3343 | $_{(ab)}0.5 \pm 0.3_A{}^a$ |
| | | | | | Uninjured | $5.4 \pm 0.3_A{}^a$ | - | $_{(a)}0.199 \pm 0.302_A{}^a$ | $4.9 \pm 0.2_B{}^b$ | 0.3817 | $_{(ab)}0.5 \pm 0.4_A{}^a$ |

741
742
743

[1] For each experimental condition, growth morphology and population type, parameters of the Geeraerd model bearing different subscripts (no lowercase letters in common) are significantly different ($P \leq 0.05$)

[2] For each inactivation support, experimental condition and population type, parameters of the Geeraerd model bearing different subscripts (no uppercase letters in common) are significantly different ($P \leq 0.05$)

[3] For each inactivation support, growth morphology and population type, parameters of the Geeraerd model bearing different superscripts (no lowercase letters in common) are significantly different ($P \leq 0.05$)