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Abstract

This study aimed at quantifying the impact of the concentration of four commercial sanitizers and temperature on mold spores inactivation. The sanitizers were based on the following fungicide molecules, ethanol (ARVO 21 SR), active chlorine (ARVO CLM 600), hydrogen peroxide (Nocolyse Food) and triamine (P3 Topax 960). Food plant spores were produced under a moderate water stress, 0.95 a_w and dry-harvested to simulate airborne spores responsible for contamination in the food industry. First, *Aspergillus flavus*, *Cladosporium cladosporioides*, *Mucor circinelloides*, and two *Penicillium commune* isolates were tested against the sanitizers at 20°C and at a concentration recommended by the manufacturers. Overall, *A. flavus* was the less resistant species. Second the effects of concentration and temperature were assessed on the most resistant species, i.e., *P. commune* UBOCC-A-116003 (ARVO 21 SR and P3 Topax 960), *P. commune* UBOCC-A-112059 (ARVO CLM 600), and *M. circinelloides* (Nocolyse Food). With the exception of ARVO 21 SR, the observed inactivation kinetics were downward concave. The time necessary to obtain 4 log reduction, t_{4D}, was estimated by means of the Weibull model. At 20°C and at the recommended concentration by the manufacturers, t_{4D} (min) for the most resistant strains were equal to 2.14 (ARVO 21 SR), 7.35 (ARVO CLM 600), 39.3 (Nocolyse Food) and 82.8 (P3 Topax 960). T_{4D} was increased at lower concentrations and temperatures. These effects were more pronounced for ARVO 21 SR, t_{4D} were about 10 fold and 20 fold the above reported value, 2.14 min, at 8°C and by diluting the sanitizer by a 10:8 factor, respectively. The least effect of temperature, 3 fold, was shown for ARVO CLM 600, while concentration of P3 Topax 960 had no significant effect on t_{4D} within the recommended utilization range.

1. Introduction

Among the large supply of disinfectant products available, the selection will depend on the effectiveness, safety, cost and corrosive effect on the surfaces (Lopez et al., 2002; Wirtanen et al., 2001; Wirtanen and Salo, 2003). The effectiveness of sanitizers in Europe is evaluated according to standards set by a European Committee for Standardization (CEN), and expressed by the logarithmic reduction. The fungicidal effect is validated when at least 3 log and 4 log reductions of viable spores are achieved on carriers and in liquid suspension, respectively. *Aspergillus brasiliensis* (ATCC 16404) is the standard strain for assessing antifungal efficacy of sanitizers (European Standard 1650, 2019). Sometimes *Cladosporium cladosporioides* is tested as an additional species. This species was shown to be particularly sensitive to chlorine dioxide (Wen et al., 2017), benzalkonium chloride, peracetic acid, quaternary ammonium and sodium hypochlorite (Bernardi et al., 2018). An efficient disinfection should be based on the "worst case scenario", i.e., the most resistant species isolated from food plant environment or spoiled products. An extensive study assessed the fungicidal effect of 15 disinfectants against 25 fungal contaminants commonly found in bread and cheese manufacturing, (Bundgaard-Nielsen and Nielsen, 1995). It was not possible to determine which species was the most resistant because resistance depended on the disinfectant. A recent review on the effect of disinfectants on the inactivation of mold spores relevant to the food industry reported a great variation in resistance inter and intra species (Visconti et al., 2021a).

It is also important that laboratory tests reproduce as far as possible real conditions. In the food industry, especially in bakeries and dairies, food contamination is due to airborne fungal spores. All European standards recommend the use of fungal spores produced under optimal conditions and re-suspended into an aqueous solution. Since the pioneer work of Nickerson et al. (1981), it was reported that hydration modify drastically the physiological state of native (dry) fungal spores. For this reason, a dry-harvesting protocol of fungal spores was developed (Dao and Dantigny, 2009). Very recently, it was reported that all *P. commune* UBOCC-A-112059 dry-harvested spores produced under a moderate water stress, i.e., "food plant" spores, remained viable after a 10 min treatment to 3% hydrogen peroxide, whereas all spores produced under the standardized protocol, i.e., "laboratory" spores, were inactivated, inactivation greater than 4.1 log, (Visconti et al., 2021b). In that study, the impact of the physiological state on the inactivation of *Aspergillus flavus*, *C. cladosporioides*, *Mucor circinelloides*, and two *P. commune* isolates by disinfectant molecules was assessed. In the present study, the "food plant" physiological state only of these species were tested against four different commercial sanitizers based on ethanol, active chlorine, hydrogen peroxide and triamine.

Recommended range of temperature, concentration and contact time are provided by manufacturers on product labels and technical sheets. Sanitizers are usually tested at 20°C. Few studies reported the effect of temperature on their efficacy. Pereira et al. (2013) reported a significantly lower inactivation rate constant at 4°C than at 21°C for spores of *Aspergillus terreus*, *Cladosporium tenuissinum* and *Phoma glomerata* treated by 3 mg/l free chlorine. Wen et al. (2017) reported also a significant effect of temperature at 10 and 27°C on the inactivation rate constants for spores of *Penicillium* sp., *Trichoderma* sp. and *Cladosporium* sp. treated by 2 mg/l chlorine dioxide. In order to provide recommendations to the food industry, the main objective of the present study was to assess the impact of temperature and

concentrations on the inactivation kinetics of the most resistant species for each commercial sanitizer.

2. Material and methods

2.1. Molds

The studied molds were provided by the Université de Bretagne Occidentale Culture Collection (UBOCC, https://www.univ-brest.fr/ubocc/) and were originally isolated from spoiled dairy products (Visconti et al., 2021b). Molds were maintained on Potato Dextrose Agar (PDA) medium at 4°C.

2.1. Spores production

"Food plant" spores were produced from mycelium grown on PDA at 0.950 a_w for 7 days at 25°C. Water activity was adjusted by substituting a part of the water with an equal weight of glycerol. The relative amount of glycerol was 20.6% (w/w) to obtain PDA media at 0.950 a_w. Spores were harvested mechanically without contact with any liquid solution (dry-harvest) according to Dao and Dantigny (2009).

2.2. Sanitizers

The fungicidal activity of four commercial sanitizers was tested in this study (Table 1). The recommended fungicidal concentration of each active substance, i.e., 60% ethanol, 0.24% active chlorine, 7.9% hydrogen peroxide, and 0.125% triamine, were tested on all isolates at

116 20°C. For the most resistant isolate of each sanitizers, two additional concentrations at 20°C
117 and two temperatures (8 and 15°C) at the recommended fungicidal concentration were tested.
118 Sanitizers were diluted with sterile distilled water.

119

120 *2.3. Inactivation testing procedures*

121

122 Spores treatment started by applying 10 ml of sanitizer solution to the dry spores on the lid.
123 For *M. circinelloides*, this volume was reduced to 5 ml as fewer spores were harvested. Prior
124 to the first sampling, the suspension (biocide + spores) was transferred to a Falcon tube and
125 homogenized by vortexing for 10 s. For each contact time tested, biocides were neutralized by
126 diluting ten-fold the suspension in neutralizing (Table 1). Experiments were carried out in
127 triplicate. The initial amount of "food plant" spores (N_0) was evaluated by counting on a
128 Malassez cell according to Dao et al. (2008). The method was based on the principle that the
129 application of biocide does not affect the morphology of the spores during the time of the
130 experiment so it is possible to count under the microscope all the spores treated including
131 inactivated ones.

132

133 *2.4. Viability assessment*

134

135 After neutralization, the treated spores were grown on PDA at 25 °C for 3 days by spreading
136 100 µl of sample and four subsequent decimal dilutions. For ethanol based disinfectant,
137 spores were grown for 7 days. Due to their faster development, *M. circinelloides* spores were
138 grown for 1 day. Only counts (N) in the range of 10 to 100 colonies per plate were

139 considered. Inactivation of spores was expressed as the logarithmic reduction factor, $\log_{10}$
140 (N/N_0).

141

142 *2.5. Model fitting*

143

144 Non-linear regressions were performed using SlideWrite 5.0 (Advanced Graphics Software,
145 Inc., Carlsbad, CA, USA). The Weibull model was used to model the impact of concentration
146 and temperatures on kinetics inactivation. The model equation (Dao et al., 2010) was
147 expressed as:

148 $$log_{10}\left(\frac{N}{N_0}\right) = -\frac{1}{2.303}\left(\frac{t}{\alpha}\right)^{\beta} \quad (1)$$

149 where α is the scale parameter (h) and β the shape parameter (dimensionless).

150 By substituting t for t_{4D}, and $\log_{10}(N/N_0)$ for 4, the time to obtain 4 log reduction was:

151 $$t_{4D} = e^{\left(\frac{Ln9.2}{\beta}+Ln\alpha\right)} \quad (2)$$

152 The 95% confidence interval for t_{4D} was calculated from the 97.5% confidence intervals for α
153 and β, using Eq. (2).

154

155

156 **3. Results**

157

158 *3.1. Fungicide activity of different sanitizers against dairy contaminants*

159 Fungicide efficacy for the different commercial sanitizers on the studied molds was shown on
160 Table 2. *A. flavus* never exhibited the strongest resistance, for all sanitizers and for the contact
161 times chosen more than 4 log reductions were obtained. *C. cladosporioides* exhibited a greater
162 resistance than *A. flavus* to hydrogen peroxide, however *M. circinelloides* was even more
163 resistant than *C. cladosporioides*. Accordingly, *M. circinelloides* was selected as the more
164 resistant species to hydrogen peroxide, 0.5 log reduction only for 20 min contact time. While
165 the two *P. commune* isolates were the less resistant species to hydrogen peroxide, they shown
166 a great resistance to the other sanitizers than the three other species. The *P. commune*
167 UBOCC-116003 isolate was the most resistant to commercial sanitizers that contained ethanol
168 and triamine. Although no significant difference in the log reduction was noticed between the
169 two *P. commune* isolates was shown, the UBOCC-A-112059 was selected for assessing the
170 impact of temperature and concentration of the chlorine-based sanitizer. The two *P. commune*
171 isolates were clearly more resistant to 0.24% chlorine for 1 min contact time (less than or
172 equal to 0.6 log inactivation) than *M. circinelloides* (more than 4.1 log inactivation).

173

174 *3.2. Impact of application temperature and sanitizer concentration on spores*
175 *inactivation*

176 The effect of ethanol concentration at 20°C on the inactivation of *P. commune*
177 UBOCC-116003 was shown on Figure 1. At the recommended concentration, inactivation
178 kinetics were log-linear, but at lower concentrations these kinetics appeared downward
179 concave. The time to obtain 4log reductions was increased with decreasing the ethanol
180 concentration. Similarly, the time to obtain 4log reductions was increased by decreasing
181 temperature, Figure 2. The effect of chlorine concentration at 20°C on the inactivation of *P.*
182 *commune* UBOCC-112059 was shown on Figure 3. At 0.48% active chlorine, inactivation
183 kinetics looked like log linear, but at 0.24 and 0.12%, the inactivation kinetics were clearly

184 upward concave. At 0.24% active chlorine, the shape of the curves remained upward concave
185 at 8 and 15°C, Figure 4. The effect of hydrogen peroxide at 20°C on the inactivation of *M.*
186 *circinelloides* was shown on Figure 5. The shape of the inactivation curves were upward
187 concave whatever the concentration (Figure 5) and at the recommended concentration (7.9%
188 H_2O_2) at 15 and 20°C, Figure 6. The effects of triamine concentration and temperature on the
189 inactivation of *P. commune* UBOCC-A-116003 were shown on Figure 7 and Figure 8,
190 respectively. In all cases upward concaves curves were obtained.

191 With the exception of the use of 60% ethanol at 20°C, the inactivation curves obtained
192 with ARVO 21-SR were downward concaves as demonstrated by β-values significantly less
193 than 1, Table 3. The impact of temperature on t_{4D}, time necessary to obtain 4log reduction,
194 was shown on Table 3. With the exception of the use of 0.48% active chlorine commercial
195 sanitizer at 20°C, the inactivation curves obtained with ARVO CLM-600 were upward
196 concaves as demonstrated by β-values significantly greater than 1, Table 4. The α-values
197 depended significantly on the concentration, but they were not significantly different at 15
198 and 20°C. The t_{4D} values were increased with decreasing temperature. At 20°C, the greater t_{4D}
199 (13.9 min) was obtained for the smallest concentration. With the exception of the use of 7.9%
200 hydrogen peroxide at 8°C, the inactivation curves obtained with Nocolyse Food were upward
201 concaves as demonstrated by β-values significantly greater than 1, Table 5. The t_{4D} values
202 were greater for the lowest temperature and concentration. The impact of temperature and
203 concentration on the efficacy of P3-Topax 960 based on triamine was shown Table 6. For the
204 respective concentrations recommended by the manufacturers, the t_{4D} values for the UBOCC-
205 A-116003 *P. commune* were 2.14 min and 82.8 min for the ARVO-21-SR and the P3-Topax
206 960 sanitizers, respectively. A strong effect of temperature on t_{4D} was highlighted for all
207 commercial sanitizers. At 8°C, t_{4D} was 3 fold the value obtained ay 20°C for ARVO CLM-
208 600, and more than 10 fold for the other commercial sanitizers. There is no significant impact

209 of the triamine concentration within the utilization range recommended by the manufacturer
210 of the P3 Topax 960.

211

212 **4. Discussion**

213

214 The objective of modelling was to estimate the time to reach 4 log reduction. In fact,
215 this reduction should be achieved to prove the fungicidal activity of a commercial sanitizer for
216 liquid experiments. By analogy to the study of Frison et al. (2015), this time was named t_{4D}.
217 The Chick-Watson (Watson, 1908) and the delayed Chick-Watson (Rennecker et al., 1999,
218 2001) models where used by Pereira et al., (2013); Wen et al. (2017) and Ma and Bibby
219 (2017), respectively, to describe the inactivation of fungal spores in drinking water. These
220 models are based on describing the inactivation process as a function of the variable Ct, where
221 C the concentration (mg/l) and t, the time. None of these models were therefore suitable to
222 model inactivation kinetics as function of the time, only. The simplest model that describes
223 inactivation kinetics is the Bigelow, or log linear model. As suggested by β-values
224 significantly different from 1 in most cases, our data could not be fitted with this model.

225 Many models based on three parameters were used to fit sigmoidal inactivation
226 kinetics. The modified Gompertz (Linton et al., 1995, 1996) and the log-logistic model (Chen
227 and Hoover, 2003; Cole et al. 1993) were used to describe inactivation of *Dothiorella*
228 *gregaria* and *Fusarium tricinctum* by aqueous chlorine dioxide (Chen and Zhu, 2011). The
229 biphasic inactivation model (Cerf, 1977) was used to describe inactivation of *Aspergillus*
230 section *Nigri* by sodium hypochlorite and peracetic acid (Frison et al. 2015), and *Aspergillus*
231 *brasiliensis* by peracetic acid (Scaramuzza et al. 2020).

As pointed out by Dantigny (2021), fitness is one of the major quality of a model, i.e., the model should fit the experimental data with a correct shape. None of the obtained kinetics in our study were sigmoidal. Therefore, there was no need to use a three parameters model such as those described in previous paragraph. The Weibull model with only two parameters was able to fit accurately both the observed shapes, (i.e., either downward or upward concave), with a good quality of fit as suggested by the low MSE values.

Comparison with literature data concerned dry harvested spores only because the physiological state of fungal spores affected greatly their resistance to disinfectants (Visconti et al., 2021b). In that study, *A. flavus* was the most resistant species with 0.9 log inactivation for 7.5 min contact time with 0.2% active chlorine. For the same contact time, *A. flavus* was most sensitive to 0.24% active sanitizer chlorine with an inactivation greater than 4.9 log. For 2 min contact time with 0.2% active chlorine the authors showed inactivation of 4.6±0.9 and 3.5±0.2 log for *P. commune* UBOCC-A-112059 and UBOCC-A-116003, respectively (Visconti et al., 2021b). The difference in sensibility between the two isolates was not significant. In the present study, at 0.24% active chlorine with ARVO CLM 600, inactivation was 4.3 and 4.5 log for isolate UBOCC-A-112059 and UBOCC-A-116003, respectively. The difference in sensibility between the two isolates was not significant either with the commercial product based on active chlorine.

At 3% hydrogen peroxide for 15 min, inactivation of *A. flavus* was 1.4 log (Visconti et al., 2021b). Treatment with 7.9% hydrogen peroxide from sanitizers for 20 min provided inactivation greater than 4.5 log. 3% hydrogen peroxide showed intraspecific variability between the two *P. commune* isolates. The inactivation were 0.5 and 1.5 log for isolate UBOCC-A-112059 and UBOCC-A-116003, respectively (Visconti et al., 2021b). For hydrogen peroxide based sanitizer for 20 min, inactivation was greater than 5.2 log for both isolates. *C. cladosporioides* and *M. circinelloides* were the most resistant molds to 3%

hydrogen peroxide as well as to sanitizer. *C. cladosporioides* was more resistant than *M. circinelloides* to 3% hydrogen peroxide for 15 min, 0.4 and 1.1 log inactivation, respectively (Visconti et al., 2021b). However, at 7.9% hydrogen peroxide from sanitizer for 20 min, inactivation was 0.5 and 1.4 log for *M. circinelloides* and *C. cladosporioides* respectively.

For the undiluted ARVO-21-SR, *P. commune* was the most resistant isolate with inactivation of 3.7 and 4.7 log for UBOCC-A-116003 and UBOCC-A-112059 respectively. The greater resistance of isolate UBOCC-A-116003 over isolate UBOCC-A-112059 is consistent with the observations of Visconti et al. (2020) who tested a non-commercial disinfectant 70% ethanol solution on both *P. commune* isolates. In this study the inactivation were 3.9 and greater than 5.4 log for isolate UBOCC-A-116003 and UBOCC-A-112059, respectively. The efficacy of the commercial sanitizer at 60% ethanol was almost the same than the 70% ethanol concentration. This was probably due to the addition of surfactants to the sanitizer solutions that might increase efficacy against hydrophobic spores and thereby allow lower concentrations of sanitizers to provide effective control of molds (Okull et al., 2006).

For each sanitizer, the recommended fungicidal concentrations and contact times were tested at 20°C. Although the protocol used in our study was different from the standards, the treatment recommendations were effective on the most resistant isolates except for the triamine based sanitizer. According to the supplier, the ethanol based sanitizer at 60% ethanol concentration for 1 min ensured 3 log inactivation according to the European Standard 13697+A1 (2019) in which the spores are deposited in steel coupons. According to our model estimations, t_{4D} was achieved at 2 min for the most resistant isolate. For active chlorine based sanitizer, the supplier claimed 4 log inactivation at 0.24% active chlorine for 15 min according to the European Standard 1650 (2019) in dirtiness conditions. The t_{4D} in our experimental conditions was 7 min. For hydrogen peroxide based sanitizer, the product

ensures 4 log for 2 h of contact time at 7.9% hydrogen peroxide according to the AFNOR NF T 72-281 (2014) standard. The t_{4D} was 39 min in our experimental conditions. The efficacy of triamine based sanitizer is 4 log inactivation for *C. cladosporioides* at 0.125% triamine for 30 min according to the European Standard 1650 (2019). This concentration ensured an inactivation greater than 5 log at 6 min contact time for the *C. cladosporioides* strain tested in our study. However, for *P. commune* isolate UBOCC-A-116003, the most resistant isolate, the t_{4D} was 83 min under our experimental conditions. With the notable exception of P3 Topax 960, the contact time recommended by manufacturers was about half the estimated time based on food plant spores of the most resistant species.

5. Conclusions

Inactivation kinetics of dry harvested spores by commercial sanitizers were fitted satisfactorily by the Weibull model. This model was characterized by its parsimony, (2 parameters only), its flexibility (upward and downward concave curves were fitted by the same model), its goodness of fit (low MSE), and its fitness (the shape of the curves was described correctly). In general, the obtained inactivation kinetics were upward concave. This suggested a lag time prior to inactivation, thus the recommended contact times should not be shortened. Commercial sanitizers were tested at 20°C, but at 8°C the loss of efficacy was huge. This effect should be taken into account for disinfection of surfaces in refrigerated areas. Commercial sanitizers recommended to be utilized pure should not be diluted, even at 90 or 80%. In contrast, commercial sanitizers could be utilized within the minimum and maximum recommended dilutions without any problem for the tested species. Commercial sanitizers were tested upon the “worst case” scenario, i.e., a “food plant” physiological state

306 associated with the more resistant species. In this worst case, the estimated time to reach 4log
307 reduction was about twice the contact time recommended by the manufacturers, except the
308 new sanitizer based on triamine. However, these results carried out at in the laboratory should
309 be confirmed in pilot plant scale.

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References

Agence Française de Normalisation NF T 72-281, 2014. Procédés de désinfection des surfaces par voie aérienne - Détermination de l'activité bactéricide, fongicide, levuricide, mycobactéricide, tuberculocide sporicide et virucide incluant les bactériophages.

Bernardi, A.O., Stefanello, A., Garcia, M.V., Parussolo, G., Stefanello, R.F., Moro, C.B., Copetti, M.V., 2018. Efficacy of commercial sanitizers against fungi of concern in the food industry. LWT – Food Sci. Technol. 97, 25-30.

Bundgaard-Nielsen, K., Nielsen, P.V., 1995. Fungicidal effect of 15 disinfectants against 25 fungal contaminants commonly found in bread and cheese manufacturing. J. Food Prot. 59, 268-275.

Cerf, O., 1977. Tailing of survival curves of bacterial spores. J. Appl. Microbiol. 42, 1-9.

Chen, Z., Zhu, C., 2011. Modelling inactivation by aqueous chlorine dioxide of *Dothiorella gregaria Sacc*. and *Fusarium tricinctum* (Corda) *Sacc*. Spores inoculated on fresh chestnut kernel. Lett. Appl. Microbiol. 52, 676-684.

Chen, H., Hoover, D.G., 2003. Modeling the combined effect of high hydrostatic pressure and mild heat on the inactivation kinetics of *Listeria monocytogenes* Scott A in whole milk. Innov. Food Sci. Emerg. 4, 25-34.

342

343 Dantigny, P., 2021. Applications of predictive modeling techniques to fungal growth in foods.
344 Curr. Opin. Food Sci. 38, 86-90.

345

346 Dao, T., Dantigny, P., 2009. Preparation of fungal conidia impacts their susceptibility to
347 inactivation by ethanol vapours. Int. J. Food Microbiol. 135, 268–273.

348

349 Dao, T., Dejardin, J., Bensoussan, M., Dantigny, P., 2010. Use of the Weibull model to
350 describe inactivation of dry-harvested conidia of different *Penicillium* species by ethanol
351 vapours. J. Appl. Microbiol. 109, 408-414.

352

353 European Standard n. 13697+A1, 2019. Chemical Disinfectants and Antiseptics –
354 Quantitative Non-porous Surface Test for the Evaluation of Bactericidal and/or Fungicidal
355 Activity of Chemical Disinfectants Used in Food, Industrial, Domestic and Institutional Areas
356 –Test Method and Requirements Without Mechanical Action (Phase 2, Step 2).

357

358 European Standard n. 1650, 2019. Chemical disinfectants and antiseptics - Quantitative
359 suspension test for the evaluation of fungicidal or yeasticidal activity of chemical
360 disinfectants and antiseptics used in food, industrial, domestic and institutional areas - Test
361 method and requirements (Phase 2, step 1)

362

363 Frison, L., Sobrero, S., Fernandez, V., Basílico, M.L.Z., 2015. Susceptibility of black
364 *aspergilli* conidia to industrial sanitizers. Int. Res. J. Public Environ. Health. 2, 65-69.

365

366 Linton, R.H., Carter, W.H., Pierson, M.D., Hackney, C.R., 1995. Use of a modified Gompertz
367 equation to model non-linear inactivation curves for *Listeria monocytogenes* Scott A. J. Food
368 Protect. 58, 946-954.
369
370 Linton, R.H., Carter, W.H., Pierson, M.D., Hackney, C.R., Eifert, J.D., 1996. Use of modified
371 Gompertz equation to predict the effects of temperature, pH, and NaCl on the inactivation of
372 *Listeria monocytogenes* Scott A heated in infant formula. J. Food Protect. 59, 16-23.
373
374 López, V.L., Romero, R.J., Ureta, V.F., 2001. Accíon germicida in vitro de productos
375 desinfectantes de uso en la industria de alimentos. ALAN. 51, 376-381.
376
377 Ma, X. and Bibby, K., 2017. Free chlorine and monochloramine inactivation kinetics of
378 *Aspergillus* and *Penicillium* in drinking water. Water Res. 120, 265-271.
379
380 Nickerson, K.W., Freer, S.N., Etten, J.L.V., 1981. *Rhizopus stolonifer* sporangiospores: a wet-
381 harvested spore is not a native spore. Exp. Mycol. 5, 189-192.
382
383 Okull, D.O., Demirci, A., Rosenberger, D., Laborde, L.F., 2006. Susceptibility of Penicillium
384 expansum spores to sodium hypochlorite, electrolyzed oxidizing water, and chlorine dioxide
385 solutions modified with nonionic surfactants. J. Food Protect. 69, 1944-1948.
386
387 Peleg, M., Cole, M.B., 1998. Reinterpretation of microbial inactivation curves. Crit. Rev.
388 Food Sci. 38, 353-380.
389

390 Pereira, V.J., Marques, R., Marques, M., Benoliel, M.J., Barreto Crespo, M.T., 2013. Free
391 chlorine inactivation of fungi in drinking water sources. Water Res. 47, 517-523.
392
393 Rennecker, J.L., Mariñas, B.J., Owens, J.H., Rice, E.W., 1999. Inactivation of *Cryptosporium*
394 *parvum* oocysts with ozone. Water Res., 33, 2481-2488.
395
396 Rennecker, J.L., Kim, J.-H., Corona-Vasquez, B., Mariñas, B.J., 2001. Role of disinfectant
397 concentration and pH in the inactivation kinetics of *Cryptosporium parvum* oocysts with
398 ozone and monochloramine. Environ. Sci. Technol. 35, 2752-2757.
399
400 Scaramuzza, N., Mutti, P., Cigarini, M., Berni, E., 2020. Effect of peracetic acid on
401 ascospore-forming molds and test microorganisms used for bio-validations of sanitizing
402 processes in food plants. Int. J. Food Microbiol. 332:108772.
403
404 Visconti, V., Rigalma, K., Coton, E., Dantigny, P., 2020. Impact of intraspecific variability
405 and physiological state on *Penicillium commune* inactivation by 70% ethanol. Int. J. Food
406 Microbiol. 332, 108782.
407
408 Visconti, V., Coton, E., Rigalma, K., Dantigny, P., 2021a. Effects of disinfectants on
409 inactivation of mold spores relevant to the food industry: a review. Fungal Biol. Rev. 38, 44-
410 66.
411
412 Visconti, V., Rigalma, K., Coton, E., Dantigny, P., 2021b. Impact of the physiological state of
413 fungal spores on their inactivation by active chlorine and hydrogen peroxide. Food Microbiol.
414 100, 103850.

Watson, H.E., 1908. A note on the variation of the rate of disinfection with change in the concentration of the disinfectant. J. Hyg. 8, 536-542.

Wen, G., Xu, X., Huang, T., Zhu, H., Ma, J., 2017. Inactivation of three genera of dominant fungal spores in groundwater using chlorine dioxide: effectiveness, influencing factors, and mechanisms. Water Res. 125, 132-140.

Wirtanen, G., Salo, S., Helander, I., Mattila Sandholm, T., 2001. Microbiological methods for testing disinfectant efficiency on *Pseudomonas biofilms*. Colloids Surf. B: Biointerfaces. 20, 37-50.

Wirtanen, G., Salo, S., 2003. Disinfections in food processing – efficacy of disinfectants. Rev. Environ. Sci. Biotechnol. 2, 293-306.

Fig. 1. Kinetics inactivation of food plant spores of *Penicillium commune* strain UBOCC-A-116003 by ARVO 21 SR at 48% ethanol (■), 54% ethanol (●) and 60% ethanol (▲) at 20 °C.

Fig. 2. Kinetics inactivation of food plant spores of *Penicillium commune* strain UBOCC-A-116003 by ARVO 21 SR at 60% ethanol at 8 °C (■), 15 °C (●) and 20 °C (▲).

Fig. 3. Kinetics inactivation of food plant spores of *Penicillium commune* strain UBOCC-A-112059 by ARVO CLM 600 at 0.12% active chlorine (■), 0.24% active chlorine (▲) and 0.48% active chlorine (●) at 20 °C.

Fig. 4. Kinetics inactivation of food plant spores of *Penicillium commune* strain UBOCC-A-112059 by ARVO CLM 600 at 0.24% active chlorine at 8 °C (■), 15 °C (●) and 20 °C (▲).

Fig. 5. Kinetics inactivation of food plant spores of *Mucor circinelloides* strain UBOCC-A-112187 by Nocolyse Food at 6.32% hydrogen peroxide (■), 7.11% hydrogen peroxide (●) and 7.9% hydrogen peroxide (▲) at 20 °C.

Fig. 6. Kinetics inactivation of food plant spores of *Mucor circinelloides* strain UBOCC-A-112187 by Nocolyse Food at 7.9% hydrogen peroxide at 8 °C (■), 15 °C (●) and 20 °C

Fig. 7. Kinetics inactivation of food plant spores of *Penicillium commune* strain UBOCC-A-116003 by P3-Topax 960 at 0.125% triamine (▲), 0.175% trimaine (■) and 0.225% triamine (●) at 20 °C.

Fig. 8. Kinetics inactivation of food plant spores of *Penicillium commune* strain UBOCC-A-116003 by P3-Topax 960 at 0.125% triamine at 8 °C (■), 15 °C (●) and 20 °C (▲).

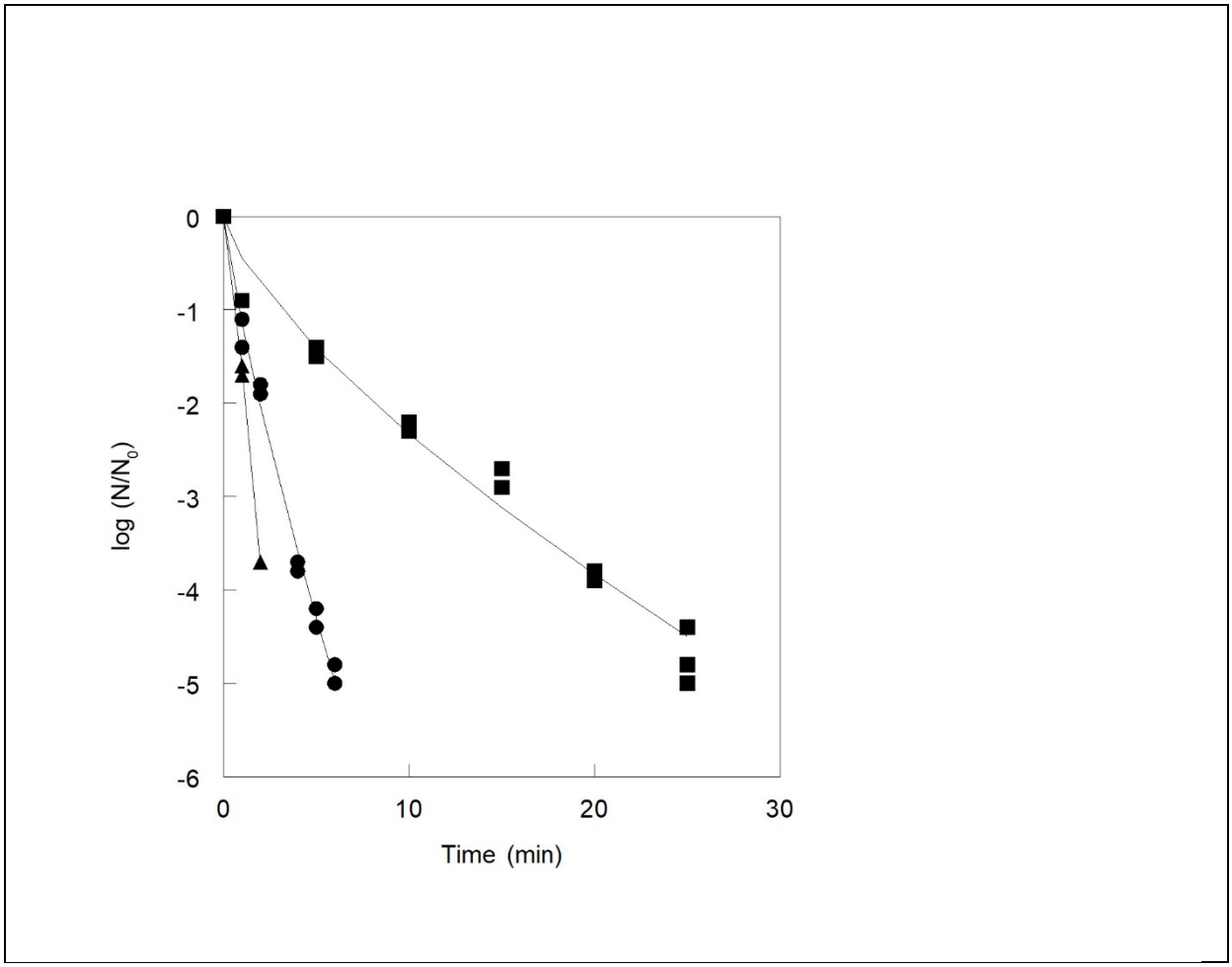

Figure 1

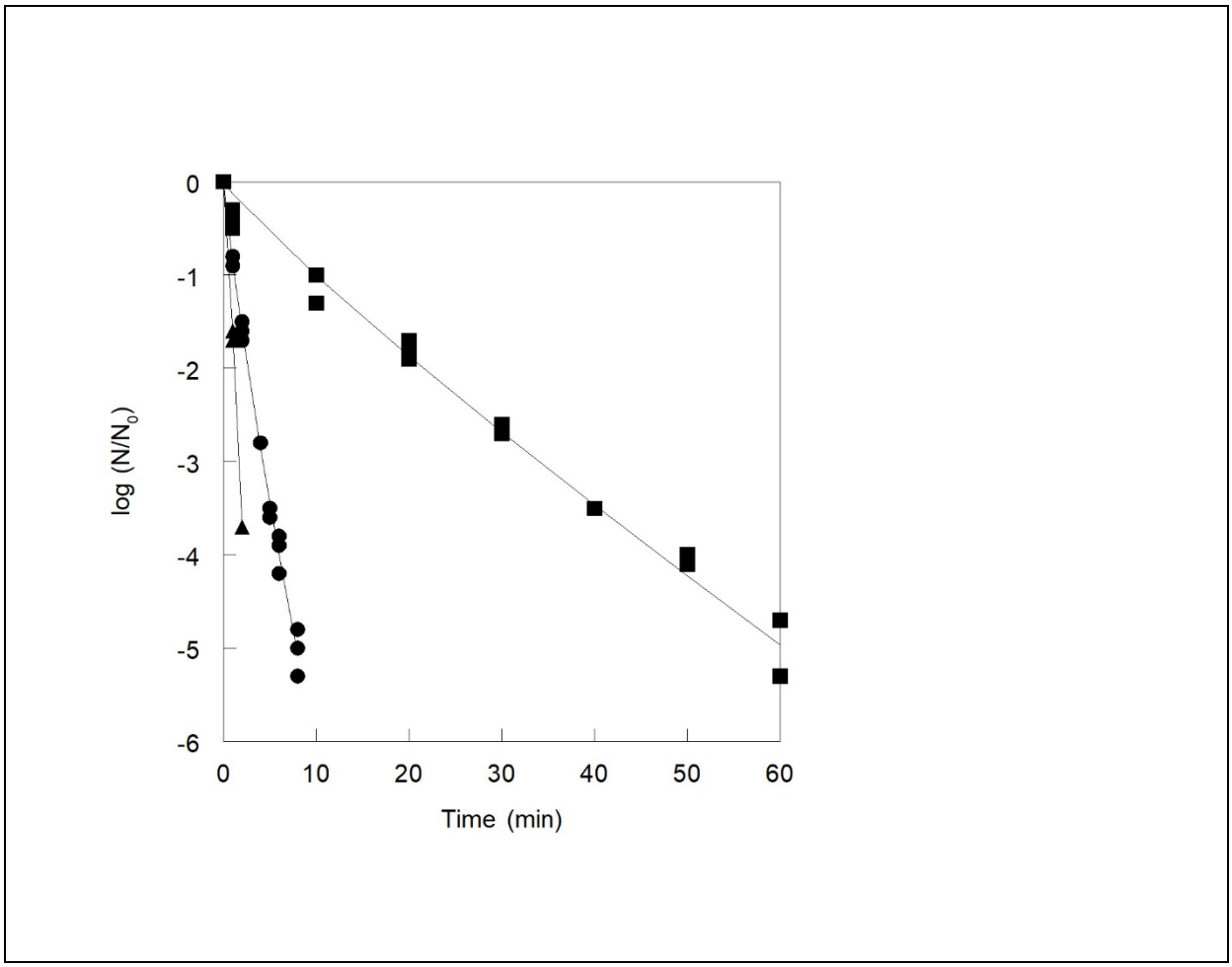

Figure 2

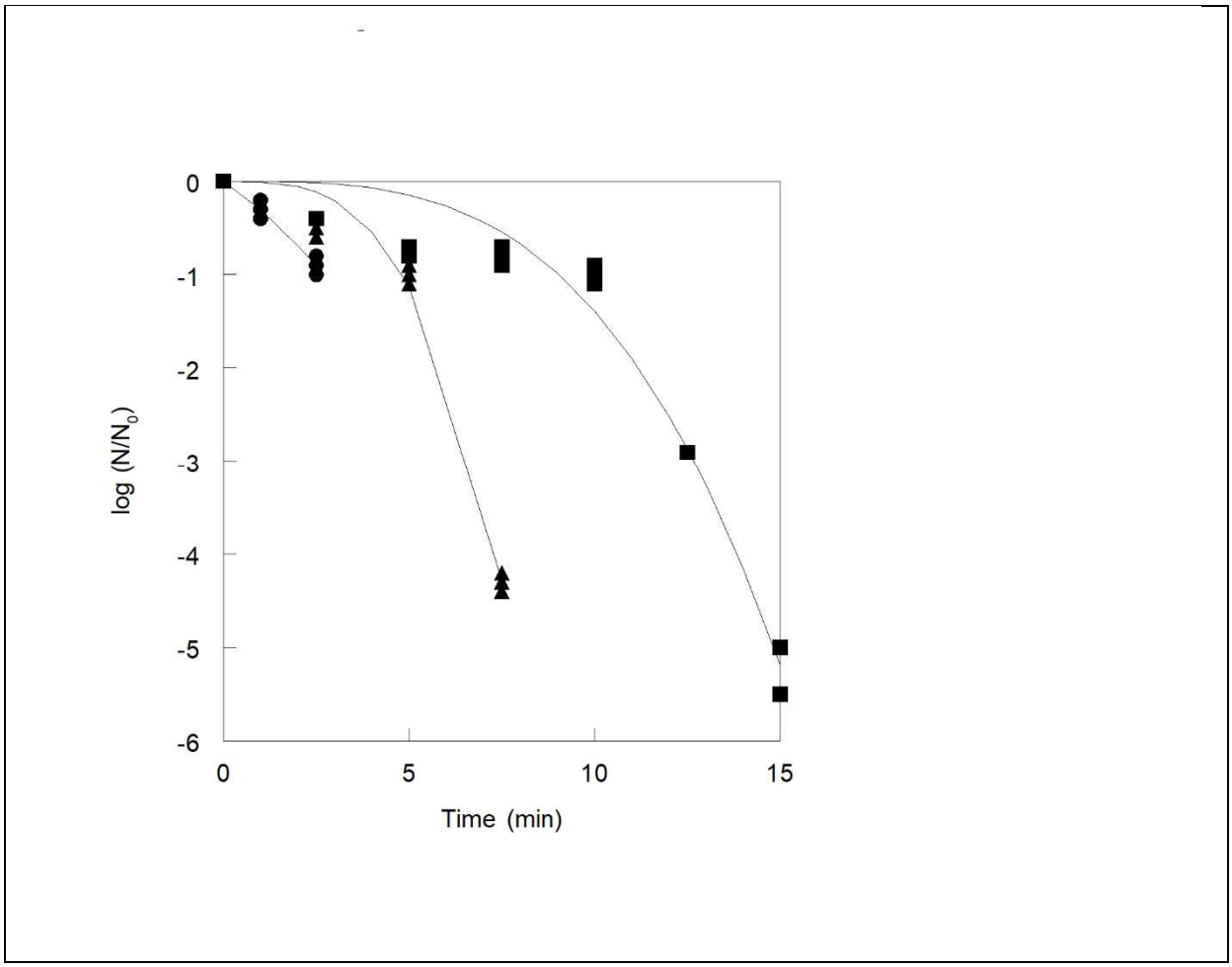

Figure 3

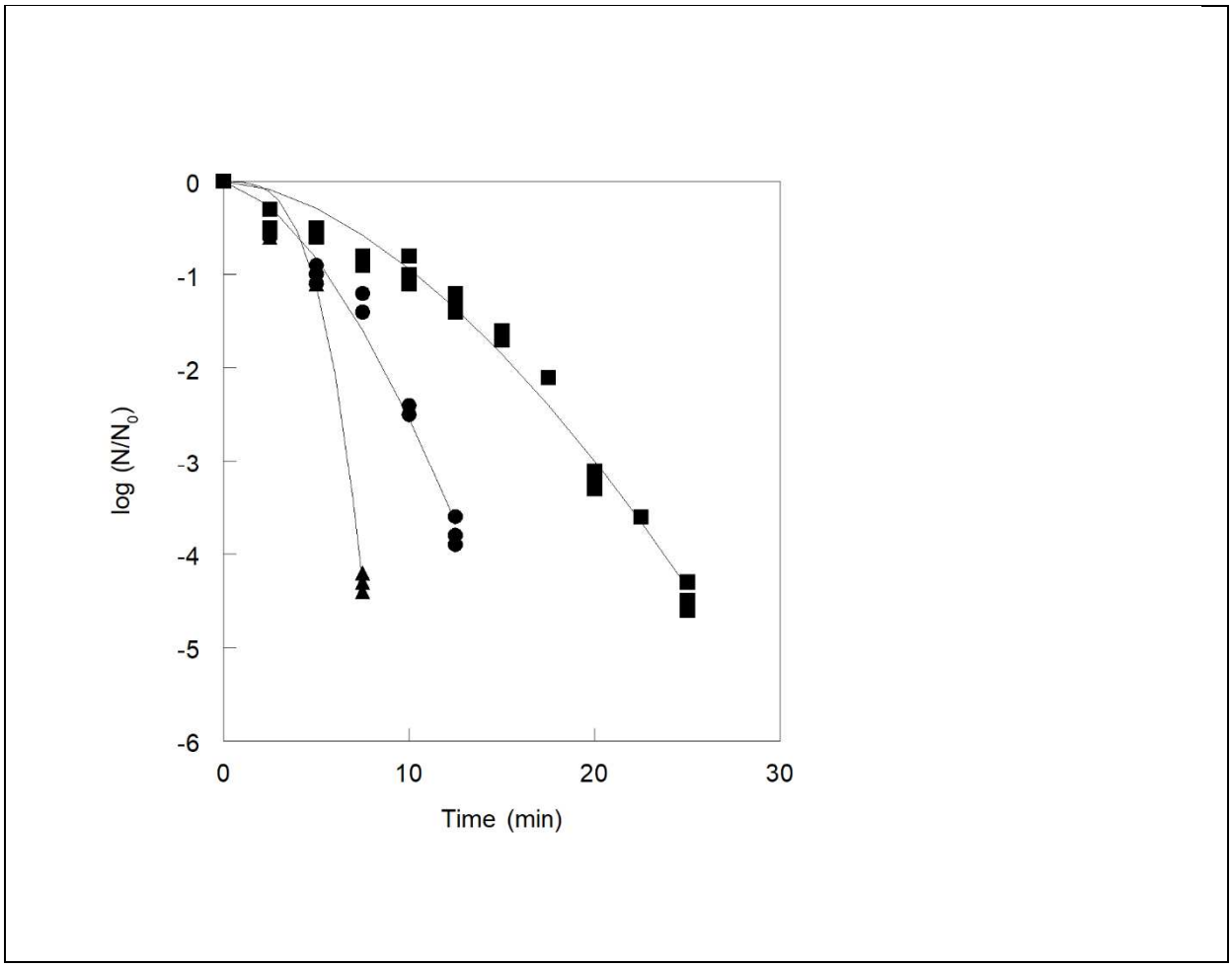

Figure 4

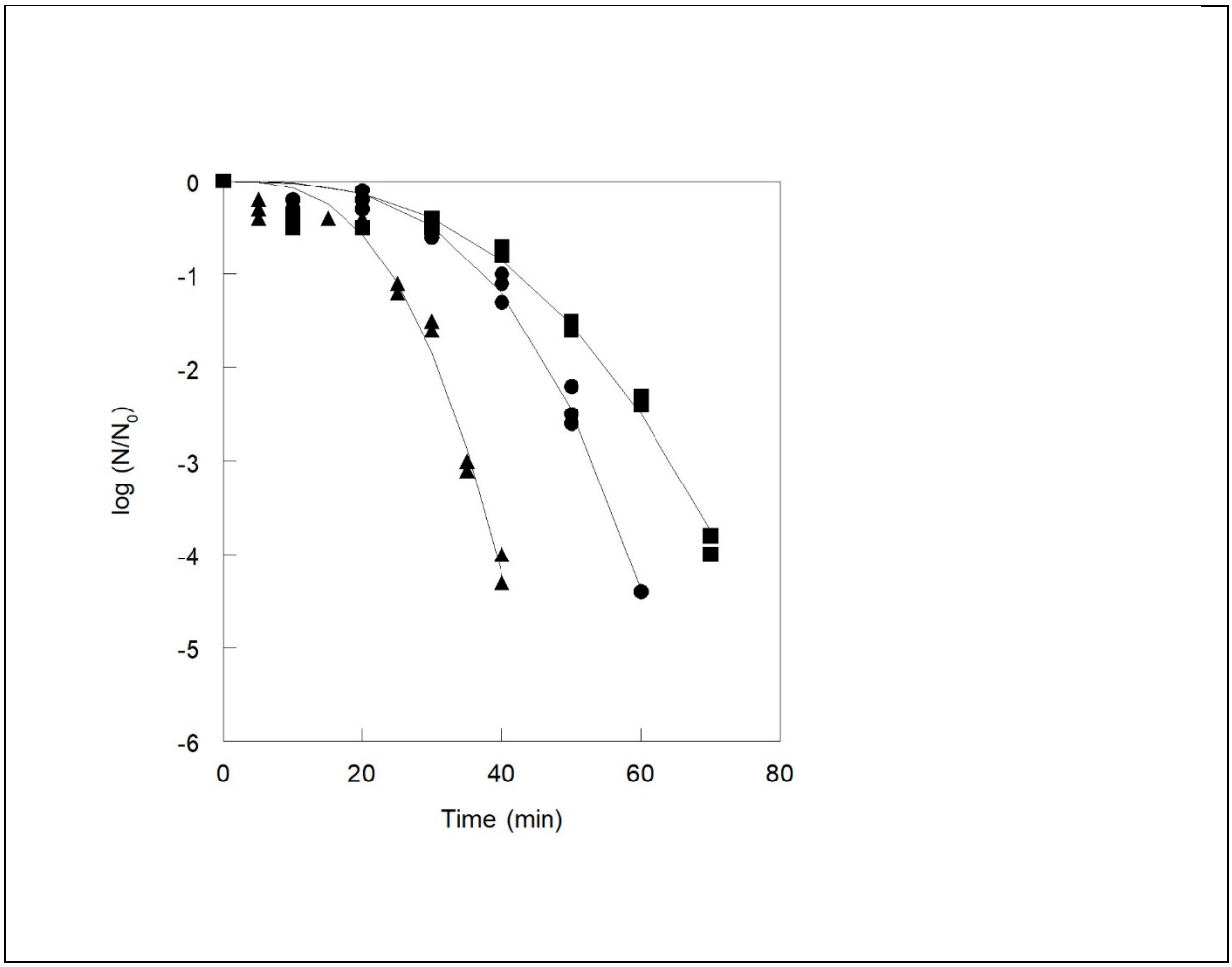

Figure 5

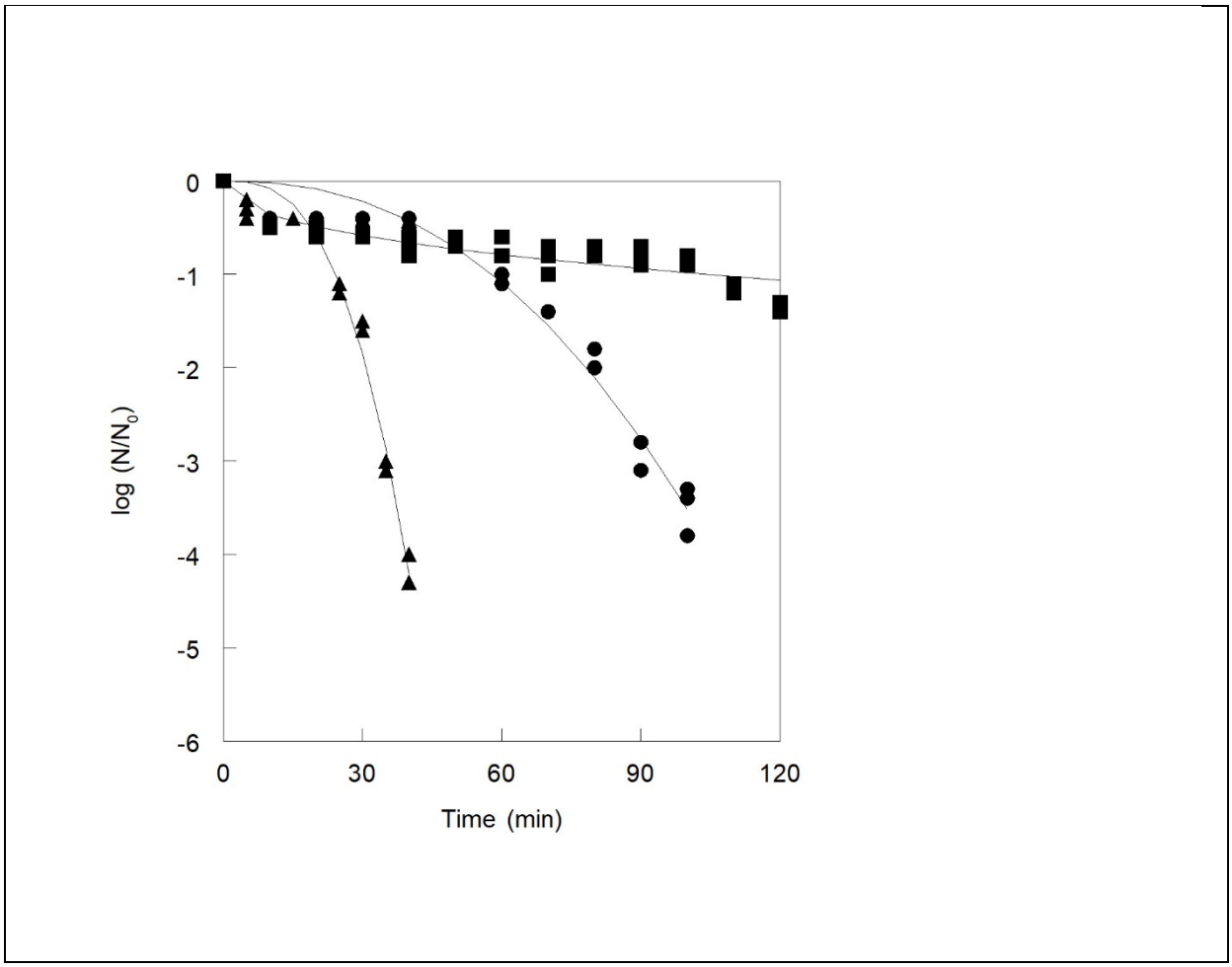

Figure 6

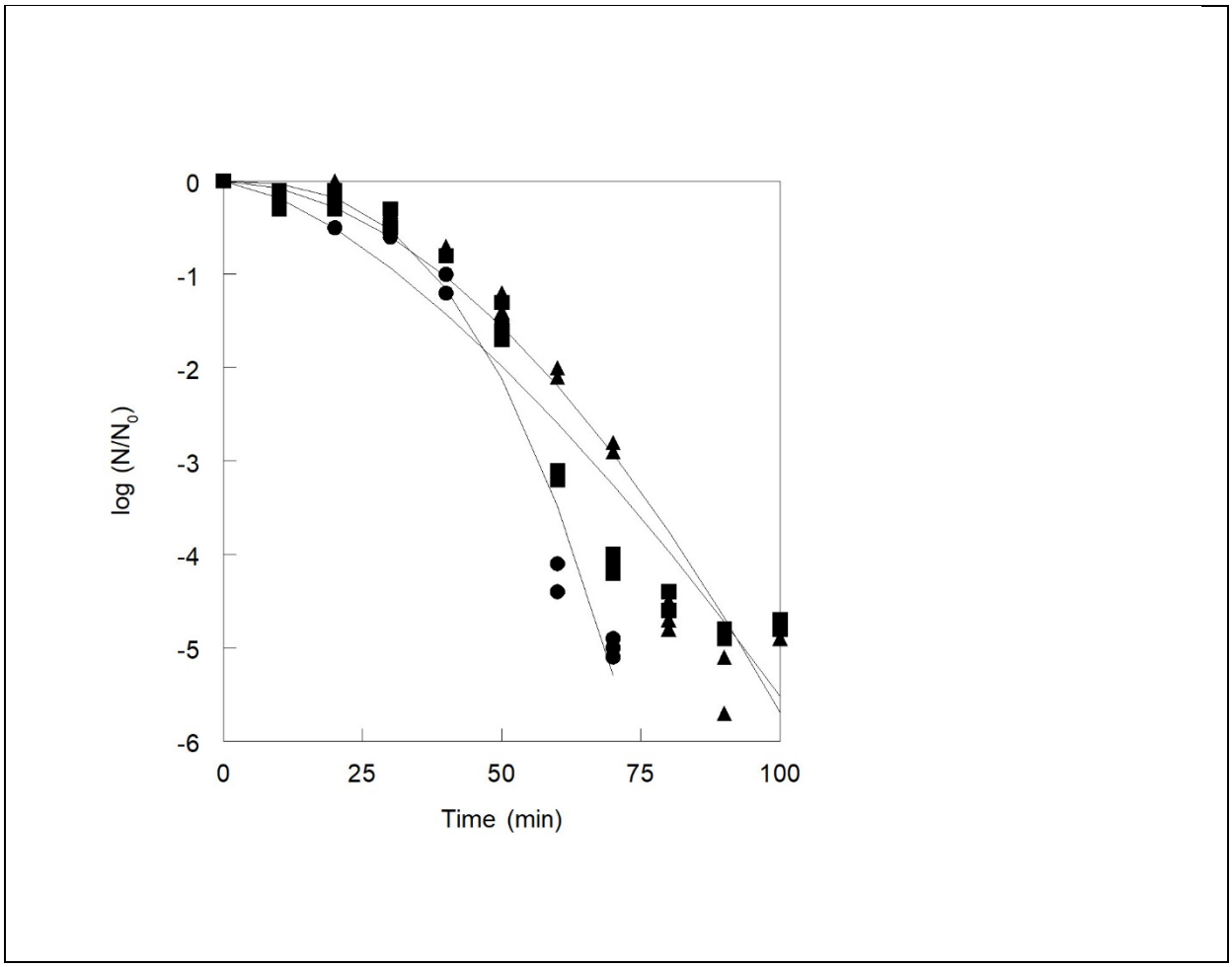

Figure 7

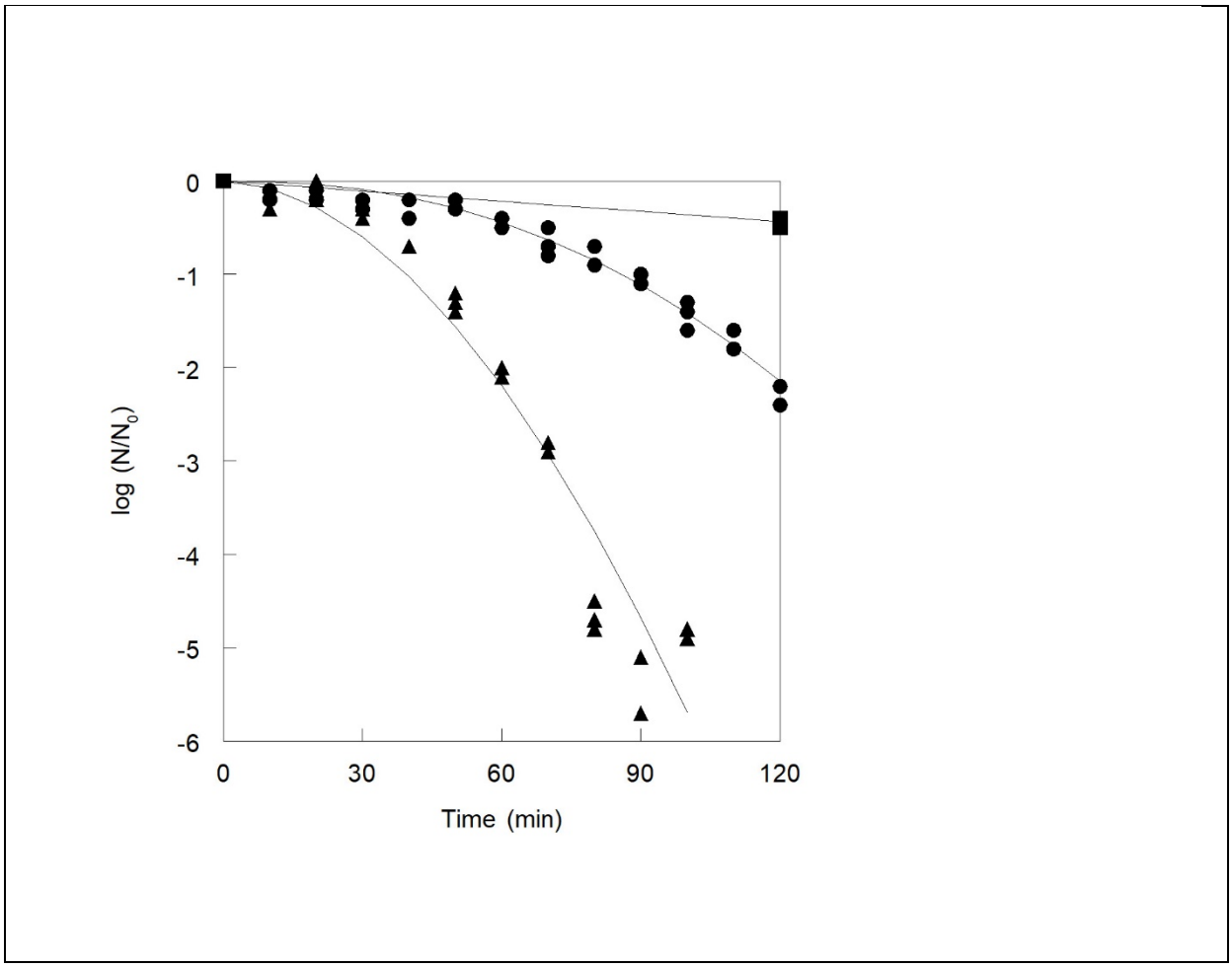

Figure 8

Table 1. List of commercial sanitizers and their associated neutralizers

Commercial sanitizer	Manufacturer	Active molecule	Neutralizers
ARVO 21 SR	Quaron, Arnas, France	Ethanol	Saline aqueous solution (NaCl, 9 g/l) containing Tween 80 (0.015% v/v)
ARVO CLM 600	Quaron, Arnas, France	Active chlorine	1% sodium thiosulfate pentahydrate diluted in saline aqueous solution (NaCl, 9 g/l) containing Tween 80 (0.015% v/v)
Nocolyse Food	Oxy'Pharm, Champigny sur Marne, France	Hydrogen peroxide	1% sodium thiosulfate pentahydrate diluted in saline aqueous solution (NaCl, 9 g/l) containing Tween 80 (0.015% v/v)
P3-TOPAX 960	Ecolab, Arcueil, France	N-(3-aminopropyl) – N –dodecylpropane – 1,3 – diamine*	Tween 80, 30 g/l with lecithin, 3 g/l diluted in saline aqueous solution (NaCl, 9 g/l)

*Triamine, Bold data: recommended manufacturer concentrations.

Table 2. Inactivation values log (N/N_0) obtained for four commercial sanitizers at the manufacturer recommended fungicidal concentration (20°C) on dry harvested spores of five spoilage molds.

Isolate	Inactivation log (N/N_0)			
	60% ethanol 2 min	0.24% active chlorine 7.5 min	7.9% hydrogen peroxide 20 min	0.125% triamine 60 min
Aspergillus flavus UBOCC-A-108066	> 5.1	> 4.9	> 4.5	4.2 (± 0.2)
Cladosporium cladosporioides UBOCC-A-111114	> 4.5	> 4.6	1.4 (± 0.1)	> 5.0
Mucor circinelloides UBOCC-A-112187	> 3.9	> 4.1	**0.5 (± 0.1)**	> 4.0
Penicillium commune UBOCC-A-112059	4.7 (± 0.2)	**4.3 (± 0.1)**	> 5.2	2.6 (± 0.1)
Penicillium commune UBOCC-A-116003	**3.7 (± 0.1)**	4.5 (± 0.1)	> 5.2	**2.0 (± 0.1)**

Bold data were used to select the most resistant species.

Table 3. Parameter estimation and 95% confidence intervals of inactivation curves fitted with the Weibull model for dry-harvested spores of *Penicillium commune* strain UBOCC-A-116003 by ARVO 21 SR.

T (°C)	Dilution factor	Ethanol (%)	α (min)	95% CI	β	95% CI	MSE	t_{4D} (min)	95% CI
8	1	60	3.86[a]	3.04; 4.68	0.888[a]	0.812; 0.964	0.034	47.0[a]	28.5; 76.7
15	1	60	0.423[b]	0.356; 0.490	0.836[a]	0.786; 0.886	0.016	6.03[b]	4.16; 8.64
20	1	60	0.325[c]	0.306; 0.345	1.18[b]	1.14; 1.22	0.001	2.14[c]	1.85; 2.46
20	10 : 9	54	0.306[bc]	0.242; 0.369	0.820[a]	0.758; 0.882	0.021	4.58[b]	2.82; 7.34
20	10 : 8	48	1.31[d]	0.900; 1.73	0.801[a]	0.708; 0.895	0.041	21.0[a]	9.63; 44.0

CI, Confidence interval. MSE, mean square error. Different superscript letters indicate significant differences at $p<0.05$ within the same column.

Table 4. Parameter estimation and 95% confidence intervals of inactivation curves fitted with the Weibull model for dry-harvested spores of *Penicillium commune* strain UBOCC-A-112059 by ARVO CLM 600.

T (°C)	Dilution factor	Active chlorine (%)	α (min)	95% CI	β	95% CI	MSE	t_{4D} (min)	95% CI
8	25	0.24	6.34[a]	5.65; 7.02	1.68[a]	1.53; 1.83	0.046	23.8[a]	18.4; 31.0
15	25	0.24	3.38[b]	2.79; 3.97	1.63[a]	1.39; 1.87	0.047	13.2[ab]	8.68; 20.9
20	25	0.24	3.75[b]	3.17; 4.32	3.30[b]	2.55; 4.04	0.071	7.35[b]	5.28; 10.9
20	25 : 2	0.48	1.36[c]	1.10; 1.62	1.20[a]	0.803; 1.59	0.006	8.67[ab]	4.08; 32.0
20	50	0.12	7.00[a]	6.12; 7.88	3.25[b]	2.66; 3.84	0.128	13.9[ab]	10.6; 19.0

CI: Confidence interval. MSE: mean square error. Different superscript letters indicate significant differences within the same column.

Table 5. Parameter estimation and 95% confidence intervals of inactivation curves fitted with the Weibull model for dry-harvested spores of *Mucor circinelloides* strain UBOCC-A-112187 by Nocolyse Food.

T (°C)	Dilution factor	Hydrogen peroxide (%)	α (min)	95% CI	β	95% CI	MSE	t_{4D} (min)	95% CI
8	1	7.9	15.2^{a}	8.43; 21.9	0.433^{a}	0.317; 0.549	0.020	2558^{a}	380; 37129
15	1	7.9	40.4^{b}	36.1; 44.7	2.31^{b}	2.00; 2.62	0.061	106^{b}	81.7; 141
20	1	7.9	18.2^{a}	16.8; 19.6	2.88^{bc}	2.56; 3.21	0.047	39.3^{c}	32.9; 48.0
20	10 : 9	7.11	29.0^{c}	27.4; 30.6	3.18^{c}	2.91; 3.45	0.020	58.3^{d}	51.4; 66.8
20	10 : 8	6.32	31.0^{c}	27.8; 34.3	2.65^{bc}	2.26; 3.04	0.053	71.8^{bd}	55.9: 95.0

CI: Confidence interval. MSE: mean square error. Different superscript letters indicate significant differences at $p<0.05$ within the same column.

Table 6. Parameter estimation and 95% confidence intervals of inactivation curves fitted with the Weibull model for dry-harvested spores of *Penicillium commune* strain UBOCC-A-116003 by P3-Topax 960.

T (°C)	Dilution factor	Triamine (%)	α (min)	95% CI	β	95% CI	MSE	t_{4D} (min)	95% CI
8	20	0.125	120^{a}	105; 135	1^{*}	1; 1	0.001	1108^{a}	948; 1263
15	20	0.125	59.6^{b}	56.0; 63.3	2.28^{a}	2.04; 2.53	0.016	158^{b}	132; 194
20	20	0.125	25.3^{c}	20.4; 30.1	1.87^{ab}	1.58; 2.16	0.233	82.8^{c}	54.2; 131
20	100 : 7	0.175	17.9^{d}	13.1; 22.7	1.48^{b}	1.22; 1.74	0.283	80.4^{bc}	43.3; 153
20	100 : 9	0.225	28.0^{c}	23.9; 32.1	2.73^{a}	2.25; 3.21	0.166	63.2^{c}	45.9; 90.5

CI: Confidence interval. MSE: mean square error.*: β-value set arbitrarily to 1. Different superscript letters indicate significant differences at $p<0.05$ within the same column.