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1 **Impact of temperature application and concentration of commercial sanitizers on**
2 **inactivation of food-plant fungal spores**

3

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16 **Keywords: Aspergillus, Cladosporium, Mucor, Penicillium, predictive mycology**

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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.

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44

45 **1. Introduction**

46

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

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

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

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

93

94 **2. Material and methods**

95

96 *2.1. Molds*

97

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

102

103 *2.1. Spores production*

104

105 “Food plant” spores were produced from mycelium grown on PDA at 0.950 a_w for 7 days at
106 25°C. Water activity was adjusted by substituting a part of the water with an equal weight of
107 glycerol. The relative amount of glycerol was 20.6% (w/w) to obtain PDA media at 0.950 a_w .
108 Spores were harvested mechanically without contact with any liquid solution (dry-harvest)
109 according to Dao and Dantigny (2009).

110

111 *2.2. Sanitizers*

112

113 The fungicidal activity of four commercial sanitizers was tested in this study (Table 1). The
114 recommended fungicidal concentration of each active substance, i.e., 60% ethanol, 0.24%
115 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:

$$151 t_{4D} = e^{\left(\frac{\ln 9.2}{\beta} + \ln \alpha \right)} \quad (2)$$

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 H₂O₂) 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 were 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).

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

238 Comparison with literature data concerned dry harvested spores only because the
239 physiological state of fungal spores affected greatly their resistance to disinfectants (Visconti
240 et al., 2021b). In that study, *A. flavus* was the most resistant species with 0.9 log inactivation
241 for 7.5 min contact time with 0.2% active chlorine. For the same contact time, *A. flavus* was
242 most sensitive to 0.24% active sanitizer chlorine with an inactivation greater than 4.9 log. For
243 2 min contact time with 0.2% active chlorine the authors showed inactivation of 4.6 ± 0.9 and
244 3.5 ± 0.2 log for *P. commune* UBOCC-A-112059 and UBOCC-A-116003, respectively
245 (Visconti et al., 2021b). The difference in sensibility between the two isolates was not
246 significant. In the present study, at 0.24% active chlorine with ARVO CLM 600, inactivation
247 was 4.3 and 4.5 log for isolate UBOCC-A-112059 and UBOCC-A-116003, respectively. The
248 difference in sensibility between the two isolates was not significant either with the
249 commercial product based on active chlorine.

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

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

261 For the undiluted ARVO-21-SR, *P. commune* was the most resistant isolate with
262 inactivation of 3.7 and 4.7 log for UBOCC-A-116003 and UBOCC-A-112059 respectively.
263 The greater resistance of isolate UBOCC-A-116003 over isolate UBOCC-A-112059 is
264 consistent with the observations of Visconti et al. (2020) who tested a non-commercial
265 disinfectant 70% ethanol solution on both *P. commune* isolates. In this study the inactivation
266 were 3.9 and greater than 5.4 log for isolate UBOCC-A-116003 and UBOCC-A-112059,
267 respectively. The efficacy of the commercial sanitizer at 60% ethanol was almost the same
268 than the 70% ethanol concentration. This was probably due to the addition of surfactants to
269 the sanitizer solutions that might increase efficacy against hydrophobic spores and thereby
270 allow lower concentrations of sanitizers to provide effective control of molds (Okull et al.,
271 2006).

272 For each sanitizer, the recommended fungicidal concentrations and contact times were
273 tested at 20°C. Although the protocol used in our study was different from the standards, the
274 treatment recommendations were effective on the most resistant isolates except for the
275 triamine based sanitizer. According to the supplier, the ethanol based sanitizer at 60% ethanol
276 concentration for 1 min ensured 3 log inactivation according to the European Standard
277 13697+A1 (2019) in which the spores are deposited in steel coupons. According to our model
278 estimations, t_{4D} was achieved at 2 min for the most resistant isolate. For active chlorine based
279 sanitizer, the supplier claimed 4 log inactivation at 0.24% active chlorine for 15 min
280 according to the European Standard 1650 (2019) in dirtiness conditions. The t_{4D} in our
281 experimental conditions was 7 min. For hydrogen peroxide based sanitizer, the product

282 ensures 4 log for 2 h of contact time at 7.9% hydrogen peroxide according to the AFNOR NF
283 T 72-281 (2014) standard. The t_{4D} was 39 min in our experimental conditions. The efficacy of
284 triamine based sanitizer is 4 log inactivation for *C. cladosporioides* at 0.125% triamine for 30
285 min according to the European Standard 1650 (2019). This concentration ensured an
286 inactivation greater than 5 log at 6 min contact time for the *C. cladosporioides* strain tested in
287 our study. However, for *P. commune* isolate UBOCC-A-116003, the most resistant isolate, the
288 t_{4D} was 83 min under our experimental conditions. With the notable exception of P3 Topax
289 960, the contact time recommended by manufacturers was about half the estimated time based
290 on food plant spores of the most resistant species.

291

292 **5. Conclusions**

293

294 Inactivation kinetics of dry harvested spores by commercial sanitizers were fitted
295 satisfactorily by the Weibull model. This model was characterized by its parsimony, (2
296 parameters only), its flexibility (upward and downward concave curves were fitted by the
297 same model), its goodness of fit (low MSE), and its fitness (the shape of the curves was
298 described correctly). In general, the obtained inactivation kinetics were upward concave. This
299 suggested a lag time prior to inactivation, thus the recommended contact times should not be
300 shortened. Commercial sanitizers were tested at 20°C, but at 8°C the loss of efficacy was
301 huge. This effect should be taken into account for disinfection of surfaces in refrigerated
302 areas. Commercial sanitizers recommended to be utilized pure should not be diluted, even at
303 90 or 80%. In contrast, commercial sanitizers could be utilized within the minimum and
304 maximum recommended dilutions without any problem for the tested species. Commercial
305 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.

310

311

312

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314

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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% triamine (■) 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 (▲).

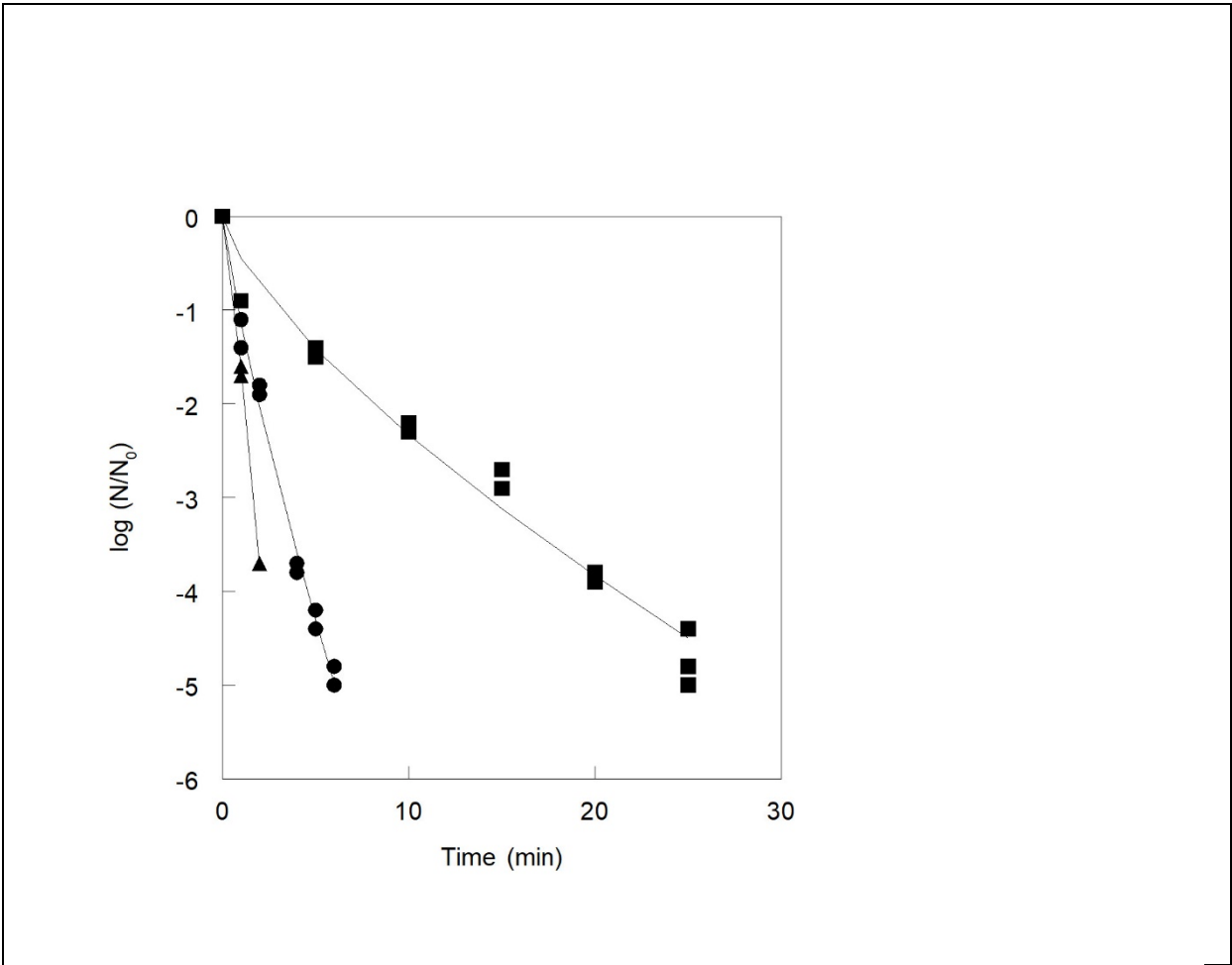


Figure 1

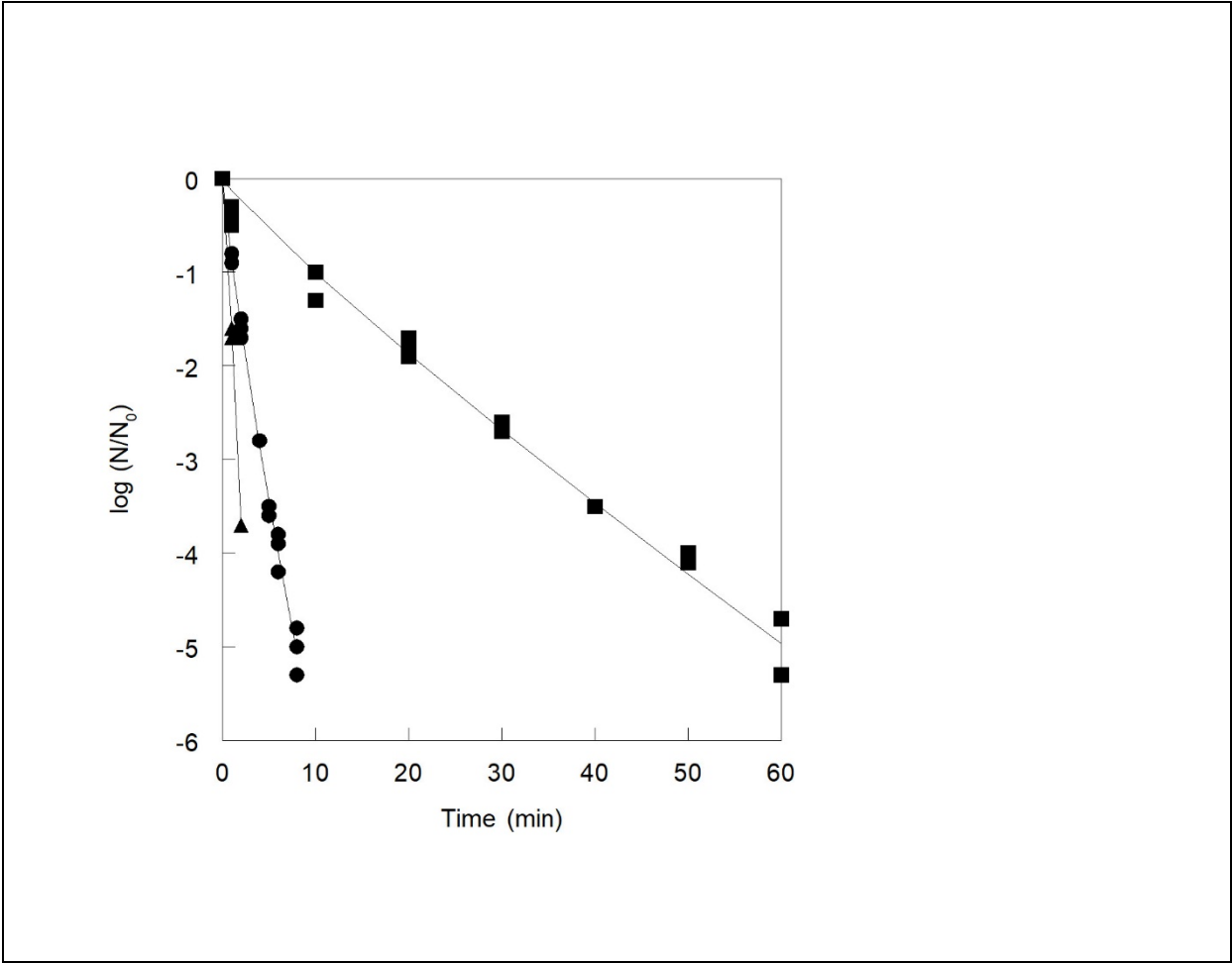


Figure 2

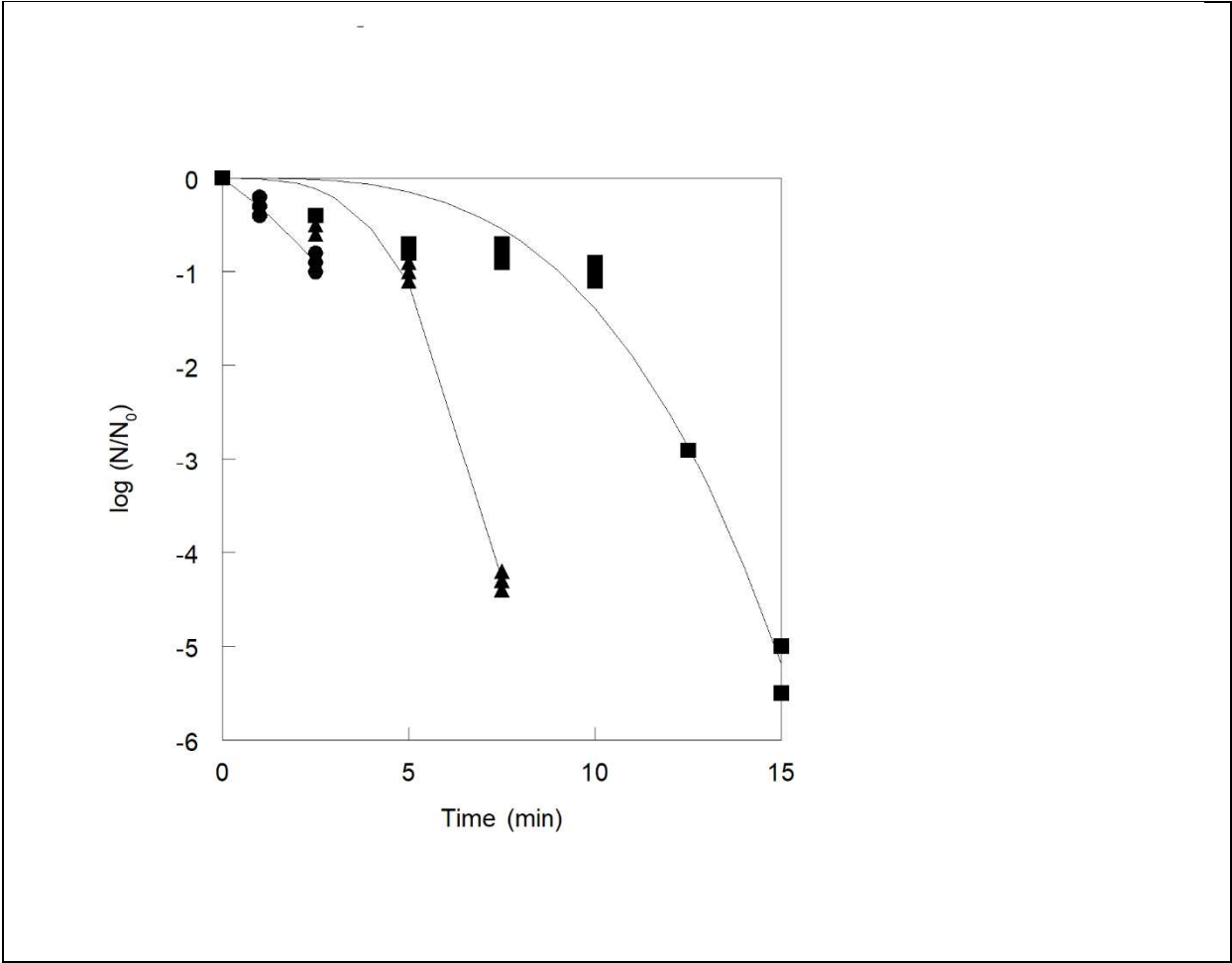


Figure 3

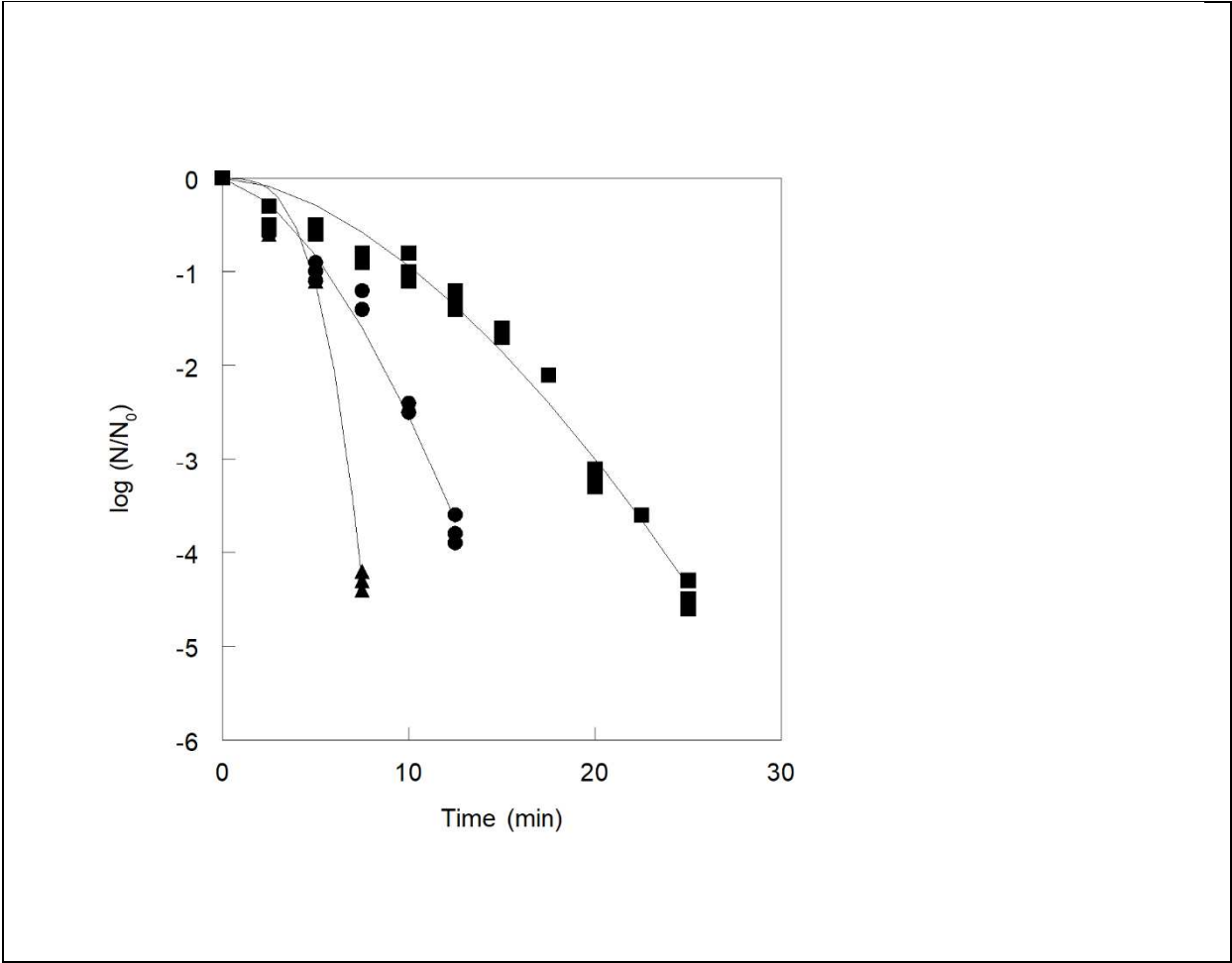


Figure 4

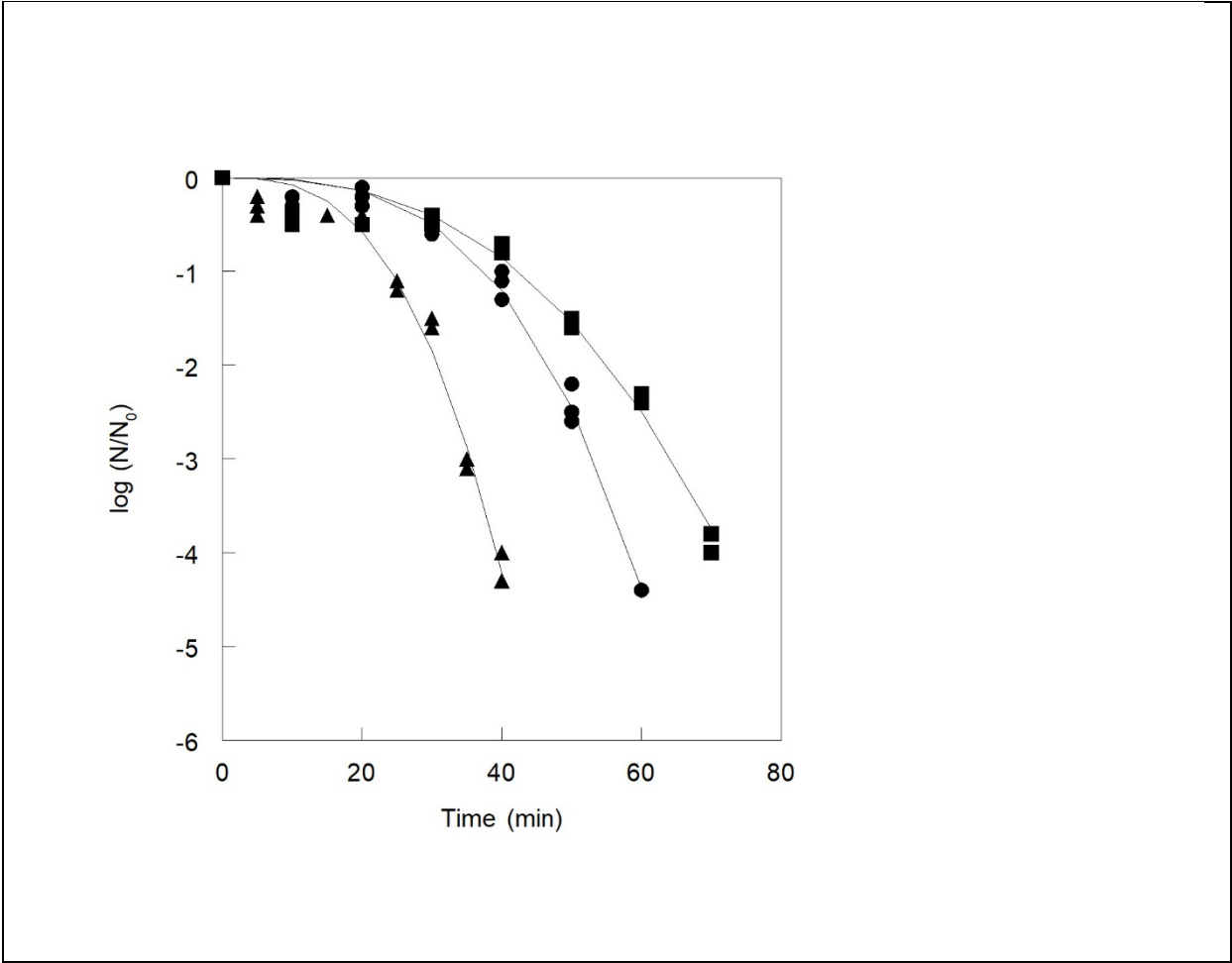


Figure 5

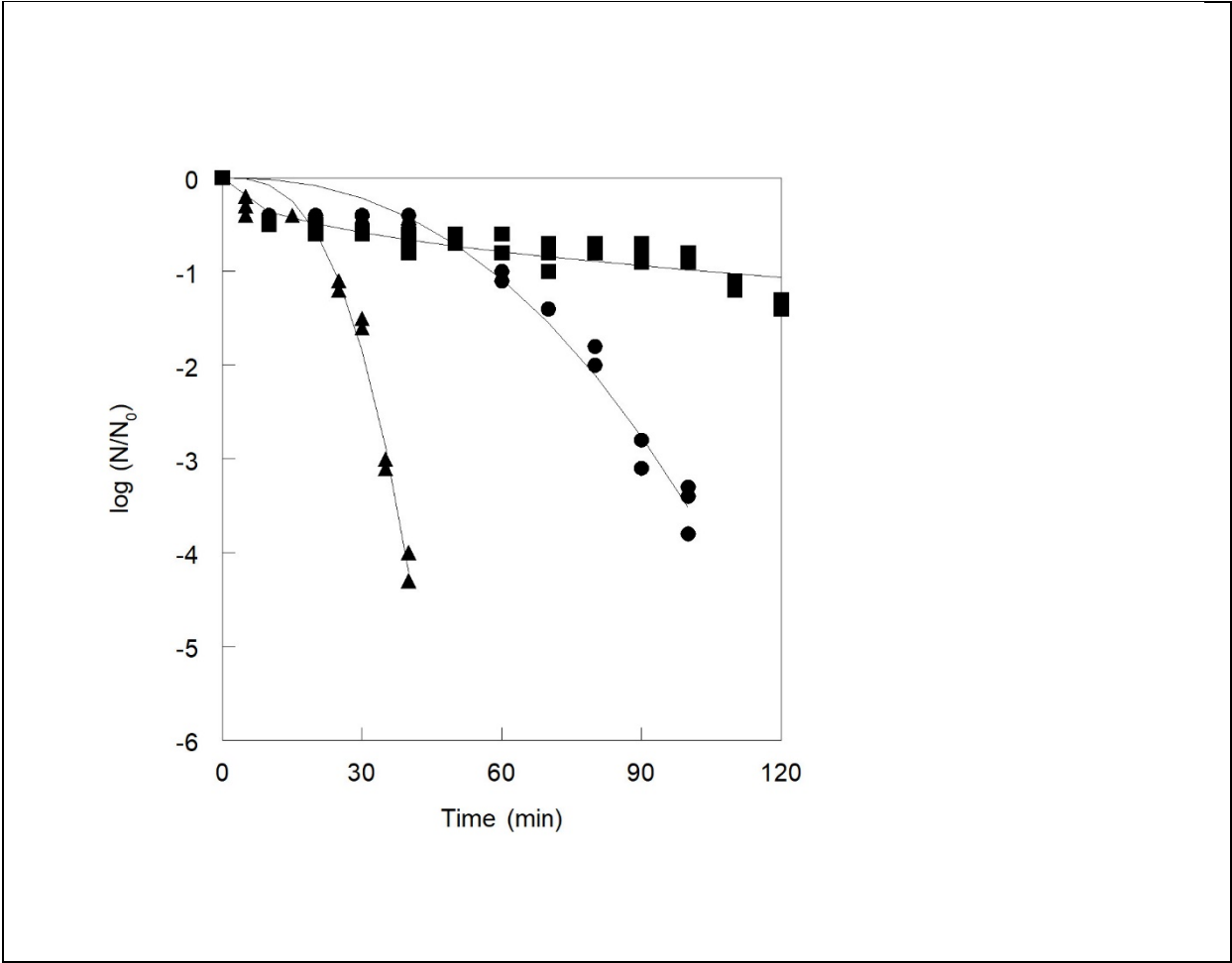


Figure 6

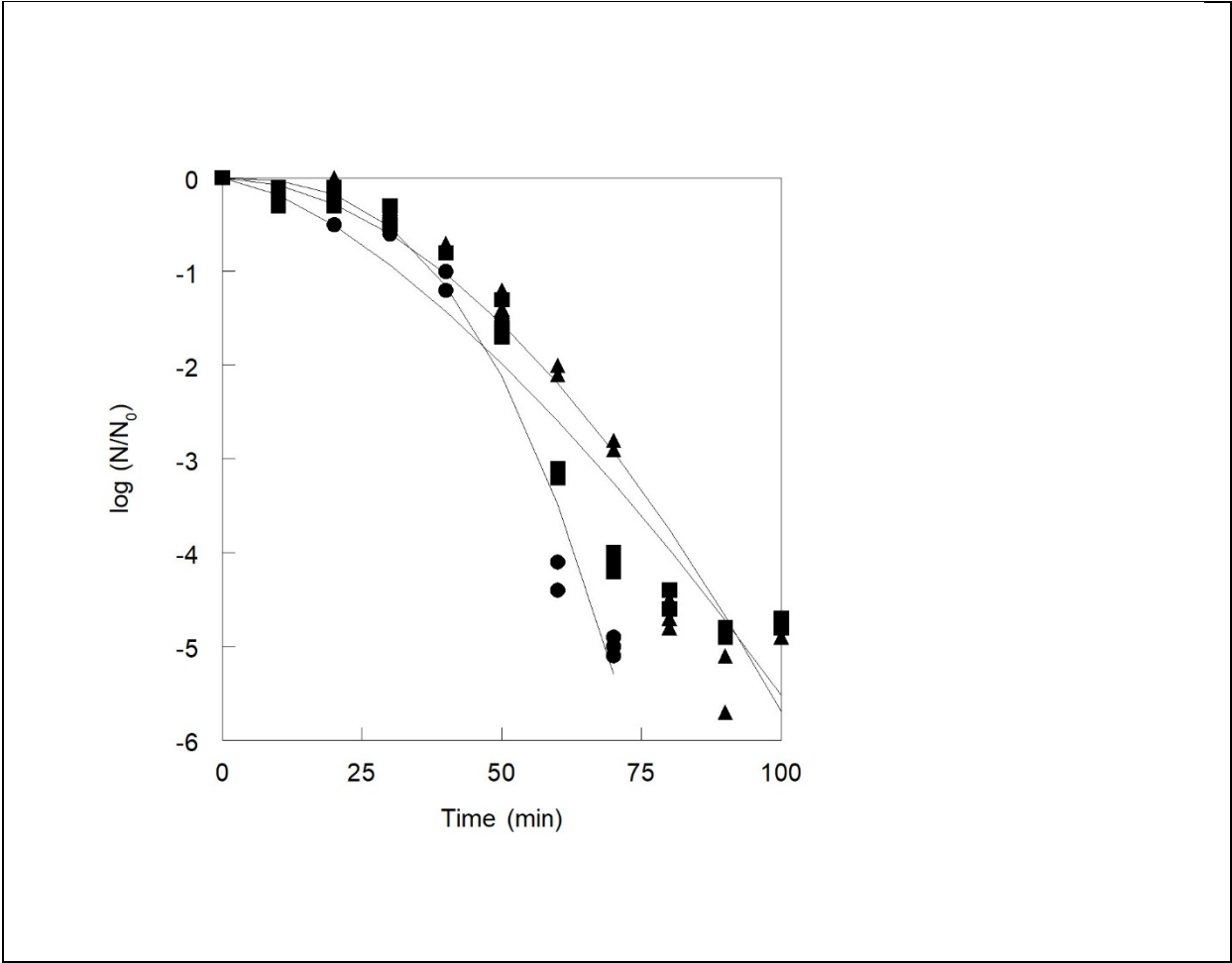


Figure 7

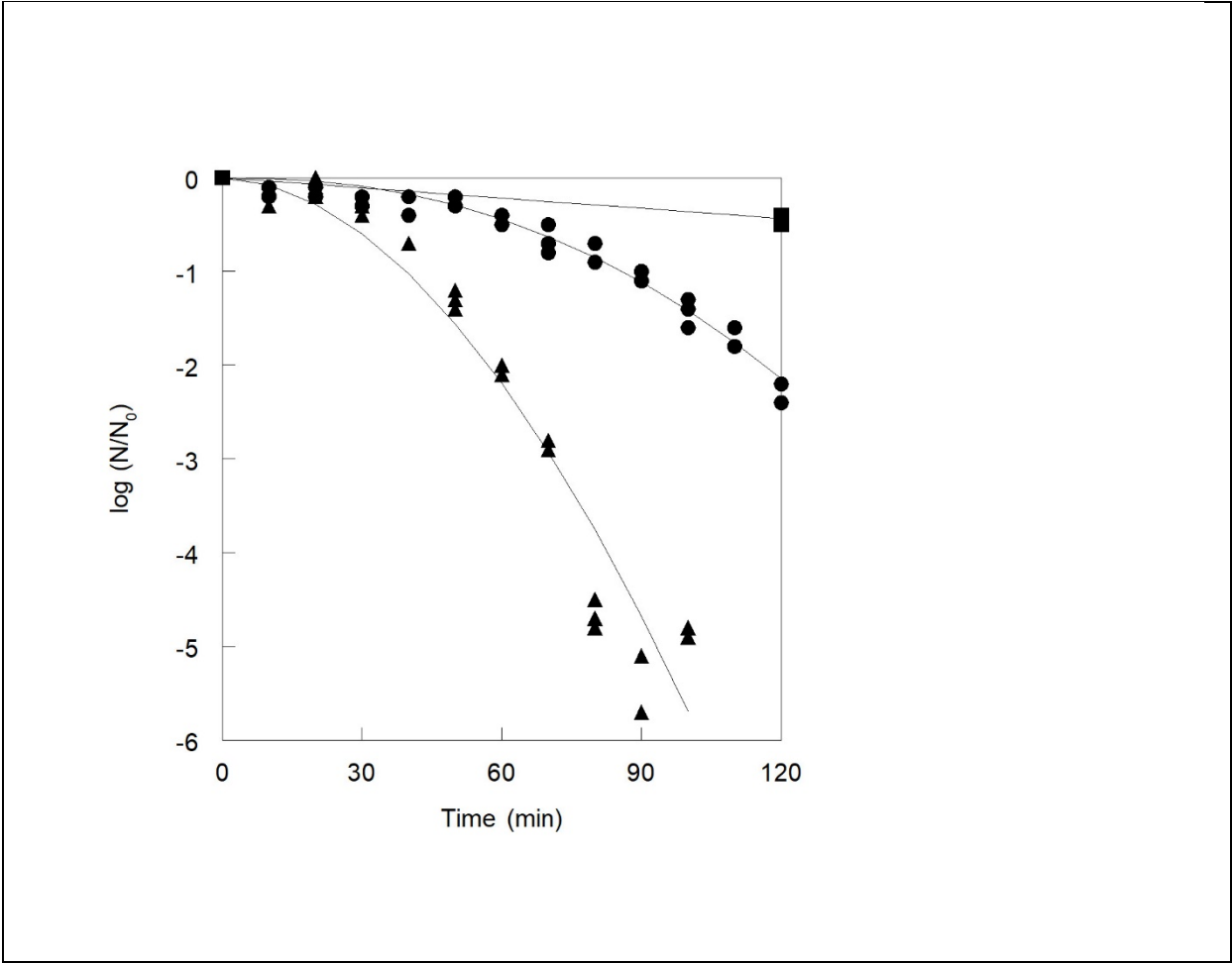


Figure 8

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/N₀) 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
<i>Aspergillus flavus</i> UBOCC-A-108066	> 5.1	> 4.9	> 4.5	4.2 (± 0.2)
<i>Cladosporium cladosporioides</i> UBOCC-A-111114	> 4.5	> 4.6	1.4 (± 0.1)	> 5.0
<i>Mucor circinelloides</i> UBOCC-A-112187	> 3.9	> 4.1	0.5 (± 0.1)	> 4.0
<i>Penicillium commune</i> UBOCC-A-112059	4.7 (± 0.2)	4.3 (± 0.1)	> 5.2	2.6 (± 0.1)
<i>Penicillium commune</i> 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.