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

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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

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

2.3. Inactivation testing procedures

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

2.4. Viability assessment

After neutralization, the treated spores were grown on PDA at 25 °C for 3 days by spreading 100 µl of sample and four subsequent decimal dilutions. For ethanol based disinfectant, spores were grown for 7 days. Due to their faster development, *M. circinelloides* spores were grown for 1 day. Only counts (N) in the range of 10 to 100 colonies per plate were considered. Inactivation of spores was expressed as the logarithmic reduction factor, $\log_{10} (N/N_0)$.

2.5. Model fitting

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

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

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

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

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

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

3. Results

3.1. Fungicide activity of different sanitizers against dairy contaminants

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

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

The effect of ethanol concentration at 20°C on the inactivation of *P. commune* UBOCC-116003 was shown on Figure 1. At the recommended concentration, inactivation kinetics were log-linear, but at lower concentrations these kinetics appeared downward concave. The time to obtain 4log reductions was increased with decreasing the ethanol concentration. Similarly, the time to obtain 4log reductions was increased by decreasing temperature, Figure 2. The effect of chlorine concentration at 20°C on the inactivation of *P. commune* UBOCC-112059 was shown on Figure 3. At 0.48% active chlorine, inactivation kinetics looked like log linear, but at 0.24 and 0.12%, the inactivation kinetics were clearly upward concave. At 0.24% active chlorine, the shape of the curves remained upward concave at 8 and 15°C, Figure 4. The effect of hydrogen peroxide at 20°C on the inactivation of *M. circinelloides* was shown on Figure 5. The shape of the inactivation curves were upward concave whatever the concentration (Figure 5) and at the recommended concentration (7.9% H_2O_2) at 15 and 20°C, Figure 6. The effects of triamine concentration and temperature on the inactivation of *P. commune* UBOCC-A-116003 were shown on Figure 7 and Figure 8, respectively. In all cases upward concaves curves were obtained.

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

4. Discussion

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

Many models based on three parameters were used to fit sigmoidal inactivation kinetics. The modified Gompertz (Linton et al., 1995, 1996) and the log-logistic model (Chen and Hoover, 2003; Cole et al. 1993) were used to describe inactivation of *Dothiorella gregaria* and *Fusarium tricinctum* by aqueous chlorine dioxide (Chen and Zhu, 2011). The biphasic inactivation model (Cerf, 1977) was used to describe inactivation of *Aspergillus* section *Nigri* by sodium hypochlorite and peracetic acid (Frison et al. 2015), and *Aspergillus 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 associated with the more resistant species. In this worst case, the estimated time to reach 4log reduction was about twice the contact time recommended by the manufacturers, except the new sanitizer based on triamine. However, these results carried out at in the laboratory should be confirmed in pilot plant scale.

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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% 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 (▲).

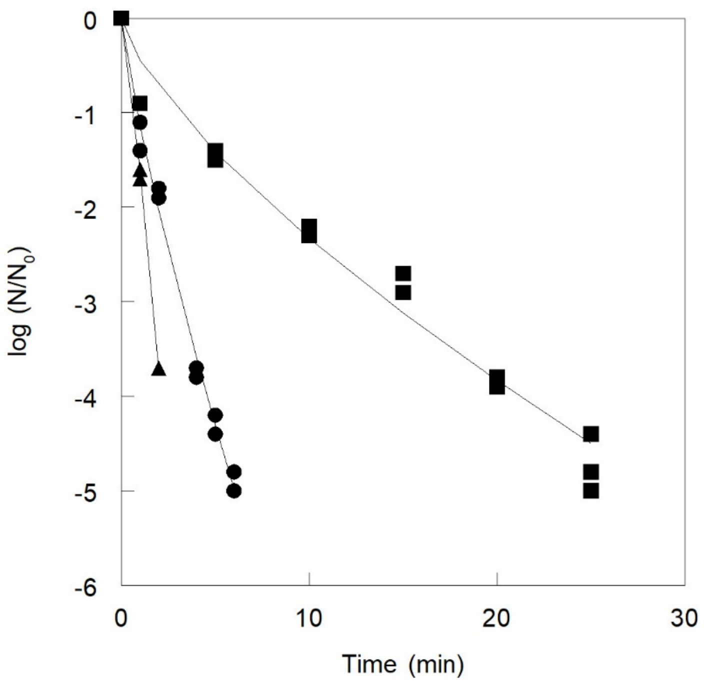


Figure 1

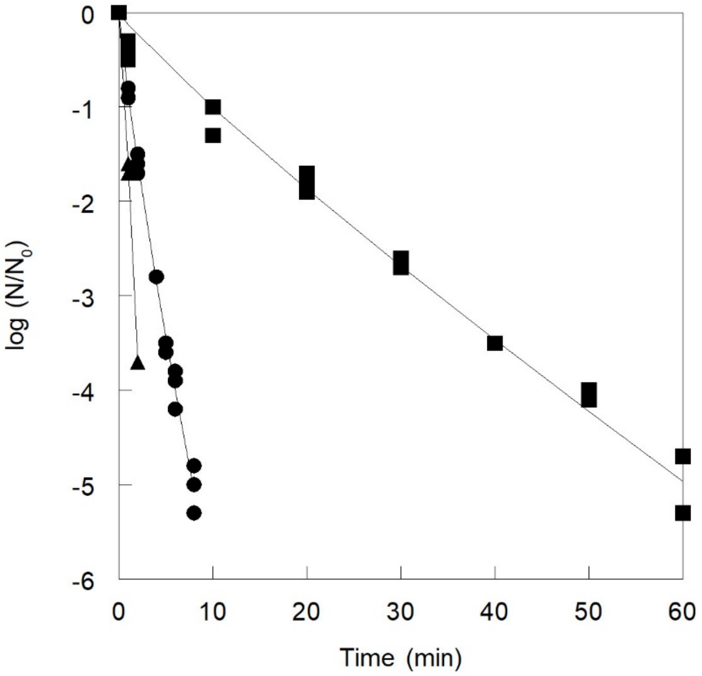


Figure 2

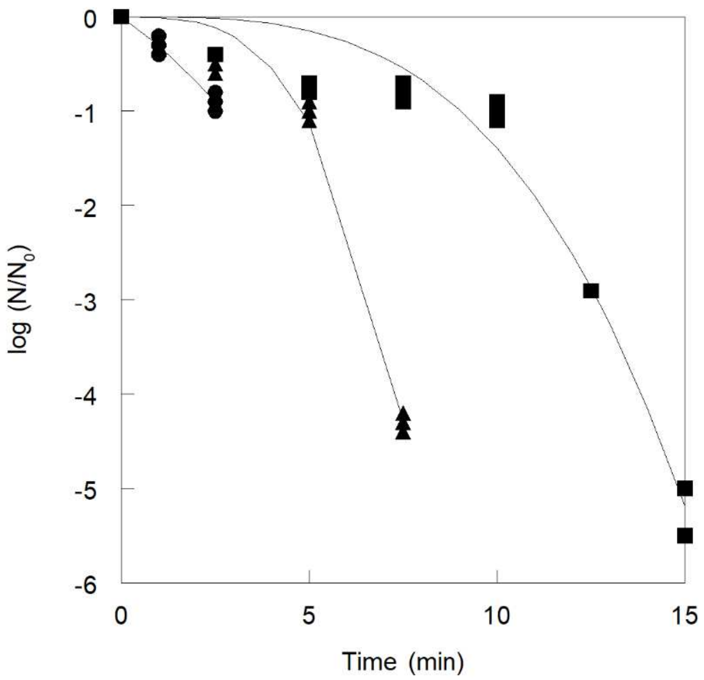


Figure 3

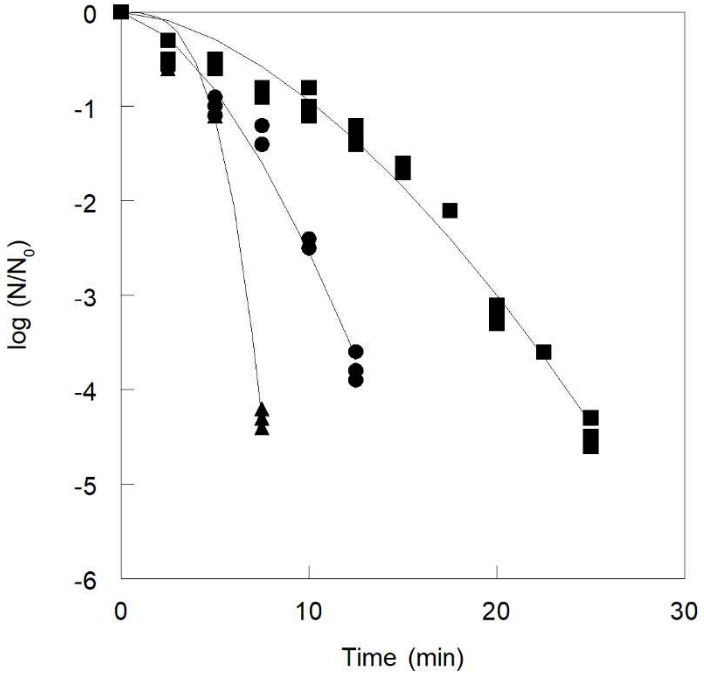
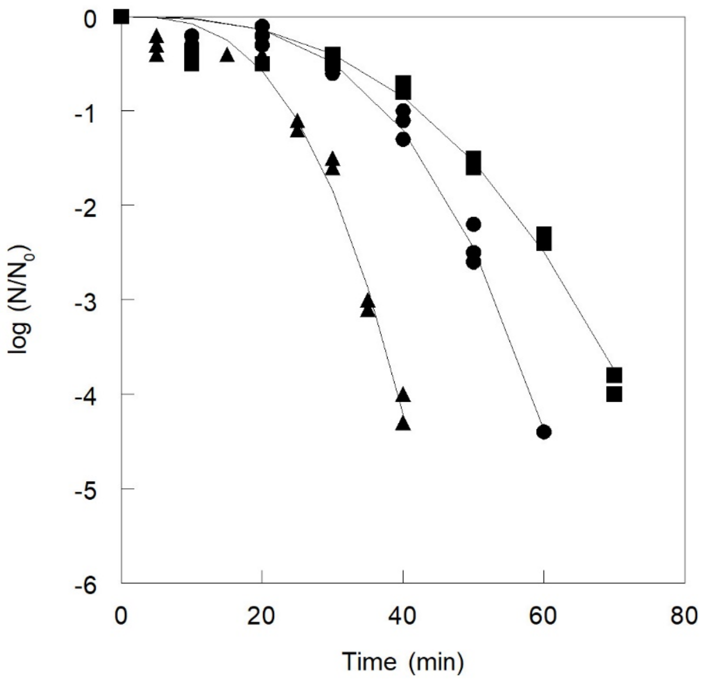


Figure 4



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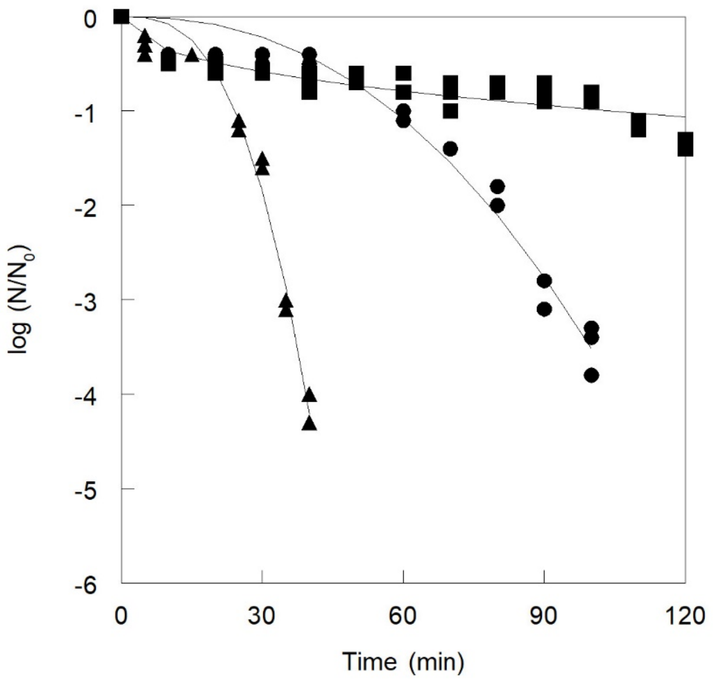

Figure 6

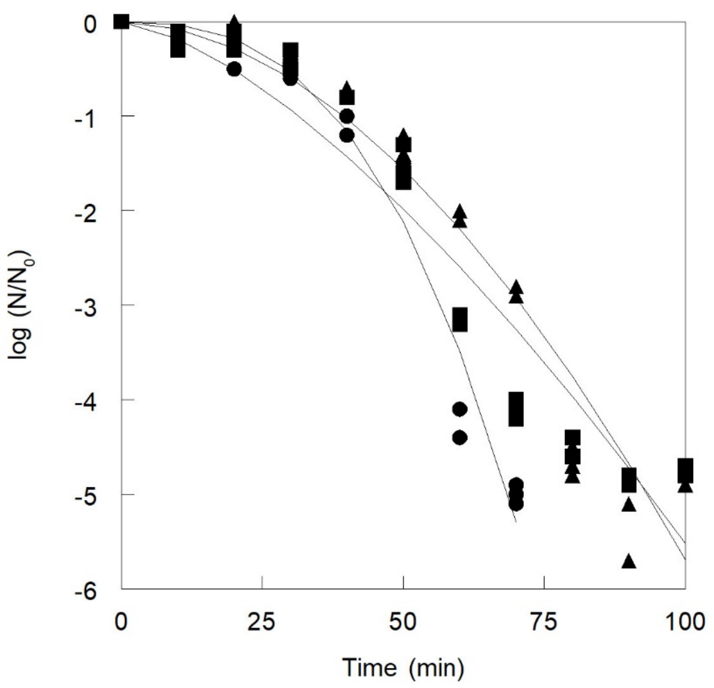

Figure 7

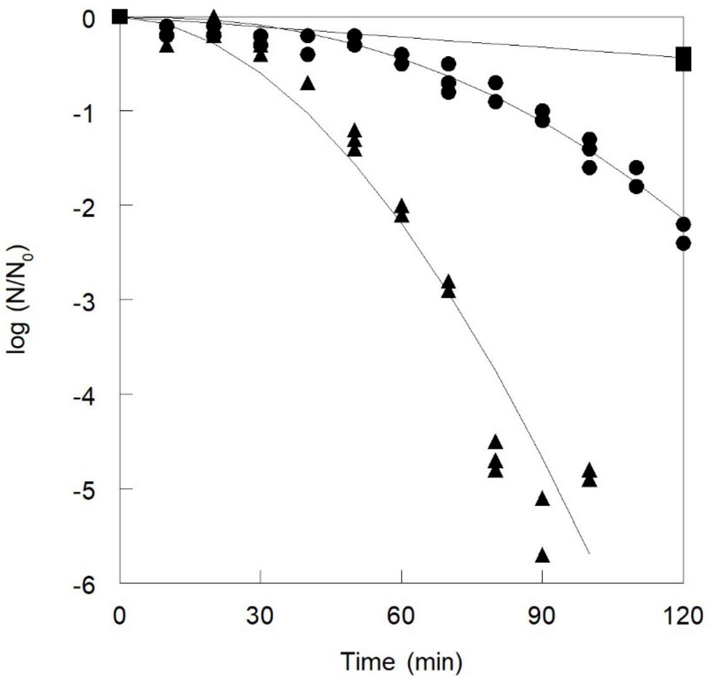

Figure 8

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.