Sterilization of food packaging by UV-C irradiation: Is *Aspergillus brasiliensis* ATCC 16404 the best target microorganism for industrial bio-validations?

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Abstract

In food industries UV-C irradiation is used to achieve decontamination of some packaging devices, such as plastic caps or laminated foils, and of those smooth surfaces that can be directly irradiated. Since its effectiveness can be checked by microbial validation tests, some ascospore-forming moulds (*Aspergillus hiratsukae, Talaromyces bacillisporus, Aspergillus montevidensis*, and *Chaetomium globosum*) were compared with one of the target microorganisms actually used in industrial bio-validations (*Aspergillus brasiliensis* ATCC 16404) to find the species most resistant to UV-C. Tests were carried out with an UV-C lamp (irradiance=127 µV/cm2; emission peak= 253.7 nm) by inoculating HDPE caps with one or more layers of spores. Inactivation kinetics of each strain were studied and both the corresponding *1D*-values and the number of Logarithmic Count Reductions (LCR) achieved were calculated. Our results showed the important role played by the type of inoculum (one or more layers) and by the differences in cell structure (thickness, presence of protective solutes, pigmentation, etc.) of the strains tested. With a single-layer inoculum, *Chaetomium globosum* showed the highest resistance to UV-C irradiation (*1D*-value=100 s). With a multi-layer inoculum, *Aspergillus brasiliensis ATCC 16404* was the most resistant fungus (*1D*-value=188 s), even if it reached a number of logarithmic reductions that was higher than those of some ascospore-forming mycetes (*Aspergillus montevidensis, Talaromyces bacillisporus*) tested.

Keywords: heat-resistant moulds*; Chaetomium globosum; Aspergillus brasiliensis* ATCC 16404*;* UV-C; packaging sterilization; bio-validations.

1. Introduction

UV-C irradiation is a non-thermal technology that gained the attention of food industries for sanitation of air, water or surfaces, since it is cost-effective, “green” (leaving no detectable residues) and with germicidal effect at short wavelengths (250-260 nm). Its ability to inactivate bacteria, yeasts, filamentous fungi, protozoa and algae is linked to RNA and DNA damages, which block their transcription and replication, leading to subsequent cellular death (Bintsis et al., 2000; Gayán et al., 2013). Despite the variable effects reported, depending on the class of microorganism considered, literature data agree on the fact that bacteria and viruses proved more sensible to UV-C light than yeasts and filamentous fungi. Among such microorganisms, those with pigmented conidia or spores were less susceptible to UV-C irradiation, maybe due to the protective effect exerted by high concentration of pigments such as melanin in their reproductive structures (Cerny, 1977; Cockell and Knowland, 1999; Esbelin et al., 2013; Wallhäußer, 1988).

UV-C irradiation for air and surface sanitization has been studied for over a century and numerous lamp disinfection systems at a wavelength of approximately 253 nm are available on the market to reduce microbial contamination of surfaces and confined areas. In clinical setting, UV-C irradiation is mainly addressed to reduce or eradicate healthcare-associated infections caused by harmful microorganisms significantly associated with increased mortality and morbidity. A recent study by Rutala et al. (2010) proved the effectiveness of UV-C treatment in eliminating vegetative bacteria on contaminated surfaces in 15 minutes and *Clostridium difficile* spores in 50 minutes in empty hospital rooms. Analogously, Escombe et al. (2009) published the first clinical trial using upper-room UV-C to prevent tuberculosis; their work was completed by Nardell et al. (2013), in order to develop design tools and guidance documents for designing effective and safe UV-C installations in real-world hospital environments. More recently, Yang et al. (2019) proved the effectiveness of an automated device utilizing UV-C irradiation to kill multidrug-resistant pathogens on surfaces and in environments of hospital rooms.

In food industries, UV-C irradiation is used to achieve decontamination of some packaging materials. Although unsuitable for glass and not easily applied to preformed tubs or lids as a result of their shape, it can be used on caps, laminated foils and those smooth surfaces that can be directly irradiated (Scholte et al., 2004). However, its effectiveness proved influenced by fluence rate (depending on the distance between surface and UV-C source) and by the presence of dust particles exerting the so-called “shadow effect” and causing non-linear behaviors in microbial inactivation (Cerny, 1977; Lippert, 1979). The effectiveness of UV-C irradiation on food packaging is assessed by microbial inactivation tests called bio-validations, as in the case of other sanitizers (e.g. hydrogen peroxide, peracetic acid, infrared and dry heat). In the field of bio-

validations, institutions such as the Institute for Processing Thermal Specialists (IFTPS) or the Mechanical Engineering Industry Association (Verband Deutscher Maschinen und Anlagenbau, VDMA) are considered a worldwide reference, providing guidance documents for microbiological validation of sterilization processes of filling machines and packaging. In the industrial practice, *Aspergillus brasiliensis* ATCC 16404 or *Aspergillus niger* ATCC 6275 are the test microorganisms suggested for assessing UV-C packaging sterilization within hygienic filling machines of Class IV (VDMA, 2005). These species were probably selected on the basis of literature data that considered them as the most UV-C resistant microbial forms (Begum et al., 2009; Ozcelik, 2007; Valero et al., 2007) able to grow in acid products (pH≤4.5) or refrigerated pasteurized products (pH>4.5). Nonetheless, it must be taken into account that, among Ascomycetes, heat-resistant moulds (HRM) (mycetes characterized by the production of ascospores very resistant to chemical and physical stresses such as heat or pressure) can also contribute to packaging contamination, and their presence could give background spoilages of various products (Dijksterhuis, 2007, Rico-Munoz, 2017), including acid foods. Until a few years ago, HRM were only occasionally searched in food and beverage packaging devices, thus being isolated from PET bottles, laminated paperboard, stretch wraps or slip sheets (Delgado et al., 2012; Rico Munoz et al., 2007; Rico-Munoz, 2017; Rico-Munoz and dos Santos, 2019; Sato and Takano, 2000). Consequently, the UV-C resistance of these microorganisms has been scarcely documented in literature, and the effect of UV-C on HRM was actually limited to papers by Begum et al. (2009) on *Aspergillus ruber* (≡*Eurotium rubrum)* in liquid medium, by Hamanaka et al. (2010) on *Paecilomyces* (≡*Byssochlamys)* sp. in stainless steel Petri dishes, and by Manns et al. (2015) on *Paecilomyces fulvus (≡Byssochlamys fulva)* in apple cider or juice.

For this reason, the aim of this work was: (i) to evaluate the resistance of three HRM to UV-C on HDPE caps, in order to find the most resistant to this kind of stress, and (ii) to compare their resistance to UV-C with that of *Chaetomium globosum* ATCC 6205, characterized by dark spores and by a strong resistance to peracetic acid (Scaramuzza et al., 2020b), and of *Aspergillus brasiliensis* ATCC 16404, one of the suggested microorganisms to assess food packaging sterilization by UV-C (VDMA, 2005).

2. Materials and Methods

2.1. Microorganisms

This study was carried out on the following fungal strains:

- *Aspergillus hiratsukae* (≡ *Neosartorya hiratsukae*) SSICA 3913, isolated from a spoiled tea beverage;

- *Talaromyces bacillisporus* SSICA 10915, isolated from heat-treated blueberries;
- *Aspergillus montevidensis* (≡ *Eurotium montevidense*) SSICA 28219, isolated from a milk-based spreadable cream;
- *Aspergillus brasiliensis* ATCC 16404, the suggested reference microorganism for validating sanitization processes on packaging or filling machines of Class IV (VDMA, 2005);
- *Chaetomium globosum* ATCC 6205, an ascospore-forming mycetes that proved resistant to other sanitizing agents such as peracetic acid (Nakayama et al., 2013; Sato and Takei, 2000; Scaramuzza et al., 2020a) and was supposedly resistant to UV-C light due to its pigmented spores.

SSICA strains were identified according to Samson et al. (2007) for *Aspergillus* with *Neosartorya* morphs, to Chen et al. (2017) for *Aspergillus* with *Eurotium* morphs, and to Yilmaz et al. (2014) for *Talaromyces* isolate. The identity of the *Aspergillus* strains with either *Neosartorya* or *Eurotium* morphs was also confirmed by molecular techniques. For *Aspergillus hiratsukae*, molecular identification was carried out as described by Berni et al. (2017). For *Aspergillus montevidensis*, DNA was extracted using a chloroform/phenol mixture. The nucleotide sequences of the internal transcribed spacers 1 and 2 region of rDNA (ITS1 and ITS2) were amplified using a SSICA internal method (unpublished data) and sequenced. The sequences obtained were compared to those available in the GenBank database (www.ncbi.nlm.nih.gov/Genbank).

2.2. Preparation of the conidial or spore suspensions

Fungal suspensions were prepared according to the protocols developed by the same authors in previous publications (Scaramuzza et al., 2020a; 2020b).

For Aspergillus brasiliensis, conidial suspensions were prepared, since this species is known to lack a teleomorphic state. After the fungus was spread on Malt Extract Agar (MEA; OXOID, Cambridge, UK), the incubation was carried out at 25 °C up to 10 days. Conidia and mycelium were harvested with a sterile loop in a 0.1% (v/v) Tween 80 solution, filtered through sterile glass wool and counted on MEA supplemented with 0.01% chlortetracycline (Sigma-Aldrich, St. Louis, MO, USA). The filtered conidial suspension was then stored at 0 °C until use.

For *Talaromyces, Aspergillus* (with either *Neosartorya* or *Eurotium* morphs) and *Chaetomium*, spore suspensions were prepared, since these species are known to produce teleomorphs and ascospores. Each strain was purified, spread on Potato Dextrose Agar (PDA, Oxoid, Cambridge, UK) and incubated at 30 °C up to 60 days to enhance ascospore production and to increase resistance (Conner and Beuchat, 1987; Dijksterhuis and Teunissen, 2004; King and Whitehand, 1990; Tournas and Traxler, 1994). Mycelium and

ascomata were collected into a 0.1% (v/v) Tween 80 solution containing sterile glass beads (3 mm diameter), shaken for 5 min using a mixer (Vortex, Continental Instruments, Amityville, NY, USA), and filtered through sterile glass wool. Spore concentrations were finally assessed by means of a Differential Interference Contrast (DIC) Microscope (Eclipse 80i, Nikon, Tokyo, Japan) to confirm that each was a single spore suspension. Filtered spore suspensions were then stored at -20 °C until use.

2.3. UV-C light inactivation tests

For sanitization tests, an UV-C lamp (230 V; 50-60 Hz; 40 W; irradiance=127 µV/cm^2; emission peak= 253.7 nm) provided with an aluminium protection screen was used. The lamp was switched on 20 min before the sanitizing treatment, to reach the maximum irradiation power. High-density polyethylene (HDPE) screw caps (diameter=30 mm; height=12 mm) were sterilized by dipping in a 96% ethanol solution, separately inoculated with each suspension and dried for one hour in sterile conditions under a laminar flow hood. All caps were spot-inoculated at the center, in order to avoid any variation in the results due to a “shadow effect” of the thread. Only the inside surface was considered for the inoculation. To assess the effect of the UV-C irradiation on one or more layers of spores, inoculations were carried out using a multi-layer (0.010 mL/spot) or a single-layer (0.10 mL/spot) deposit, with an initial concentration of the inoculated spores respectively varying from 4.2 to 5.4 Log CFU/spot or from 4.3 to 4.5 Log CFU/spot (Figure 1).

Tests were performed at room temperature positioning the caps under the light source at 2.4 cm from the inoculum (Figure 2). Treatment times varied from 20 s to 200 s (single-layer inoculum) or to 800 s (multi-layer inoculum). After each treatment, the caps were removed. Treated spores or conidia were recovered by a sterile swab, transferred to sterile PYREX® screw-cap culture tubes containing 3.0 mL of a peptone salt solution (8.5 g/l NaCl, 1 g/l tryptone), and each sample was vortexed for two minutes by a IR apparatus (Starlab, Milan, Italy). Appropriate decimal dilutions were then plated on MEA supplemented with 0.01% chlortetracycline and the colonies were counted after incubation at 25 °C up to five days (for *A. brasiliensis* ATCC 16404) or at 30 °C up to 10 days (for ascospore-forming strains). Each time/temperature combination was tested in duplicate with the single-layer inoculum and in triplicate with the multi-layer inoculum: in such case, the third repetition was carried out due to a more pronounced variability in the results obtained with the first two tranches of experiments.

2.4. Modeling the UV-C death rate curves

All UV-C death rate curves were drawn considering the logarithmic count of each microorganism at different exposure times. Unlike what happens when heat is applied to HRM, whose ascospores usually survive in a dormant state that can only be broken by either a thermal or a chemical shock (Beuchat, 1986; Dijksterhuis, 2007), UV-C irradiation did not cause activation of the ascospores of *Neosartorya*-type, *Eurotium*-type or *Talaromyces* strains studied. When both single-layer and multi-layer inocula were used, the death curves of all strains showed a non-linear behavior with a marked tailing.

2.5. Statistical analysis

Microsoft ® Excel 2013 (Microsoft, Redmond, WA, USA) was used for graphical elaboration of thermal reduction data, presented as logarithmic mean values (Table 2) or as Log N_0/N_f (Figure 3) to achieve the maximum number of Logarithmic Count Reductions (LCR) and to avoid differences due to heterogeneous initial microbial concentrations. Calculation of D-values and statistical analysis was carried out by the GInaFiT (Geeraerd and Van Impe Inactivation Model Fitting Tool, version 1.7), a freeware Add-in tool for Microsoft® Excel (https://cit.kuleuven.be/biotec/software/GinaFit). As yet done by Berni et al. (2017), both linear and non-linear models were applied as a comparison, due to a non-log-linear behavior for most of the combinations tested (Coroller et al., 2006; King et al., 1979). The goodness of fit for each elaboration was assessed using the coefficient of regression (R^2) and the Root Mean Sum of Squared Error (RMSSE). When linear models were applied, the kinetic parameters and the maximum specific inactivation rate (kmax) were obtained and the *1D*-values were calculated as 2.303/kmax for each combination, as described by Lahou et al. (2015), only considering the inactivation phase before tailing. When non-linear models were applied, the kmax or the δ parameter (scale factor) were used to determine the time needed for the first decimal reduction, being equal to the *1D*-value and all the inactivation data were considered.

To assess differences among *1D*-values in single-layer or multi-layer inoculum trials, one-way analysis of variance (ANOVA) was performed. When significant differences were detected, Fisher's least significant differences (LSD) at $p \leq 0.05$ were computed. All analyses were performed using the STATGRAPHICS® Centurion XVI v16.2.04 statistical program (Statpoint Technologies Inc., Warrenton VA, USA).

3. Results and Discussion

3.1. UV-C inactivation tests

Tests were carried out at 127 µV/cm^2 on both heat-sensitive and heat-resistant strains up to 200 s (single-layer inoculum) or 800 s (multi-layer inoculum) in order to draw inactivation curves (Figure 3) and to calculate a *D*-value for each microorganism tested (Table 1, Table 2).

As Figure 3 shows, a pronounced tailing was observed for all strains tested with both single- and multi-layer inoculum, with the exception of *A. brasiliensis* inoculated on a single-layer. Such phenomenon, already observed by Cerny (1977) and Lippert (1979), was related to the interfering action of soluble solids (when a liquid medium is treated with UV-C) or dust particles (when conidia or spores were dried and irradiated), to heterogeneous treatments, to the aggregation of microorganisms, or to the presence of resistant sub-populations (Baysal et al., 2013; Costa Menezes et al., 2019; Hijnen et al., 2006). In our tests, tailing could be attributed to the "shadow effect" exerted by spores in the upper layer of the inoculum, which blocked the UV-C irradiation and allowed a minor portion of undamaged spores to survive. Anyway, since the inactivation phase before tailing was linear, both log-linear and non-linear models were tested for a comparison of the thermal parameters.

With the single-layer inoculum, despite tailing was not considered when log-linear models were applied, the overall performance resulted in unsatisfactory statistical indices, because the regression coefficients ranged between 0.72 and 0.96, and the RMSSE was too high (0.1540-0.8069) (Table 1), thus non-linear mathematical regression models were used. When such models were applied, the regression coefficients significantly increased (varying from 0.95 to 1.00) and the RMSSE sensibly improved (0.0296-0.4021) (Table 1).

With the multi-layer inoculum, a good fitting when the log-linear model has been used was observed only for some of the strains tested (Table 2). The regression coefficients varied between 0.84 (*A. montevidensis*) and 0.97 (*A. hiratsukae*), whereas RMSSE was good (0.1157-0.2385). The differences could be attributed to the high standard deviation of some data, resulting in a suboptimal reproducibility of the tests. For this reason, log-linear models were compared with best fitting models. As Table 2 shows, using such models the regression coefficients significantly increased up to 0.98 (*A. brasiliensis*), whereas RMSSE was unchanged (0.1023-0.2690).

For both type of inoculum, the results showed that non-linear models provided the best fit to the data, with higher regression coefficients and low RMSSE values. In particular, non-linear models provided a slightly higher correlation than log-linear models (with the single-layer inoculum, the lowest R^2 were equal to 0.95 and 0.72, respectively; with the multi-layer inoculum, the lowest R^2 were equal to 0.95 and 0.84, respectively).

214 With the single-layer inoculum, a greater resistance to UV-C irradiation was registered for *C. globosum*,
215 followed by all other strains tested. *C. globosum* showed a *1D*-value close to 100 s, whereas HRM and *A.*
216 *brasiliensis* showed substantially lower *1D*-values (9.7-24.9 s) (Table 1). With the multi-layer inoculum, a
217 greater resistance to UV-C irradiation was registered for *A. brasiliensis* (*1D*-value=188 s), followed by *C.*
218 *globosum* (*1D*-value=153 s) and *T. bacillisporus* (*1D*-value=147 s). By contrast, the inactivation parameters
219 of *A. montevidensis* and *A. hiratsukae* were significantly lower (*1D*-value less than one minute).
220 Depending on the type of inoculum, the differences observed in UV-C resistance were attributed to the
221 diverse composition of the eukaryotic cells. For a single layer of spores, maybe the synergistic effect exerted
222 by walls thickness (> 0.5µm) and accumulation of solutes (trehalose and trehalose-based oligosaccharides)
223 (Friborg et al., 2004; Wyatt, 2014) could favor the survival of dark-pigmented ascospores such as those of
224 *Chaetomium globosum* rather than of black conidia such as those of *Aspergillus brasiliensis*. On the
225 contrary, with more layers and a "shadow effect", pigmentation could play a pivotal role and could therefore
226 give a greater protection to those species (e.g. *A. brasiliensis*) with high quantities of dark molecules such as
227 melanin (Pombeiro-Sponchiado et al., 2017).
228 The mean Logarithmic Count Reductions (LCR) are presented in Figure 4. When the single-layer deposit
229 was used, a marked UV-C radiation effect was observed for *A. brasiliensis* (3.8 LCR after 200 s), followed by
230 *T. bacillisporus* and *A. hiratsukae* (2.8 LCR, respectively after 80 and 160 s), *A. montevidensis* and *C.*
231 *globosum* (1.8 and 1.7 LCR, respectively after 40 and 160 s). On the contrary, when a multi-layer deposit
232 was carried out, LCR were lower and varied from 0.5 to 1.1 for all strains, except for *A. hiratsukae* that yet
233 reached 2.3 LCR after 80 s. With such inoculum, LCR values increased when longer treatment times were
234 considered, but any case UV-C radiation was not sufficient to reach three logarithmic reductions, the
235 minimum value required for such sanitizing method during bio-validations (VDMA, 2005).
236
237 During the last decade, UV-C irradiation proved effective for decontaminating fresh vegetables such as
238 blueberries (Zhou et al., 2019), pears (Li et al., 2010), oranges (Gunduz and Panir, 2013), or peppercorns
239 (Gabriel et al., 2020), but also for extending the shelf-life of products such as lime juice (Acevedo et al.,
240 2018), coconut water (Bhullara et al., 2016), or freshly squeezed turbid white grape juice (Unluturk and
241 Atilgan, 2015). UV-C irradiation was also tested for reducing *Aspergillus fischeri* (≡*Neosartorya fischeri*)
242 ascospores in apple juice at different soluble solids contents (Costa Menezes et al., 2019) and spores or
243 biofilm by *Alicyclobacillus* spp. on stainless steel or rubber surfaces (Prado et al., 2019). On the contrary, its
244 use as a sanitizing method of packaging materials was scarcely investigated: the only paper available

(Begum et al., 2009) registered a 3-log reduction after 120 s and a 3.5-log reduction after 180 s for spores of *A. niger* FRR 5664 dried onto a filter membrane and treated at 4644 J/m^2 (equal to 464,4 mWs/cm^2). Supposing a single-layer inoculum (10^6 spores were distributed onto a 40 mm diameter filter membrane), little differences (3.0 log vs. 3.8 log after 120 s, respectively) were observed comparing their LCR to those of our paper, and they could be attributed to the different strain used.

4. Conclusions

The presence of ascospore-forming moulds on packaging devices has been recently associated with background spoilage of pasteurized foods and beverages packed using aseptic filling systems, suggesting the need to reconsider the state-of-the-art concerning the test microorganisms (*Aspergillus brasiliensis* ATCC 16404 or *Aspergillus niger* ATCC 6275) used to assess the effectiveness of UV-C sterilization of packagings. Our results showed that in UV-C trials an important role is played by the type of inoculum applied: one or more layers of spores are subjected to different irradiation powers and this could lead to different behaviors and killing rates, depending on the differences in cellular structure (thickness, presence of protective solutes, pigmentation, etc.) of the strains considered. In the tests carried out on a single-layer inoculum, *Chaetomium globosum* ATCC 6205 showed the highest resistance to UV-C irradiation (*1D*-value close to 100 s) as also confirmed by the minimum number of reached LCR. On the contrary, in the tests carried out on a multi-layer inoculum *Aspergillus brasiliensis* ATCC 16404 was more resistant (*1D*-value =188 s) than any other strain tested, even with a number of LCR higher than those of some ascospore-forming mycetes (*Aspergillus montevidensis, Talaromyces bacillisporus*).

The minimum microbiological requirements suggested by the VDMA for packaging sterilized in hygienic filling machines of Class IV (LCR $\geq$ 3) do not seem achievable either with single-layer or multi-layer inocula, considering the extremely short UV-C application times (lower than 60 s) during the industrial sanitization processes. To overcome this problem, since low microbial loads are generally found in a wide range of packaging devices (Racchi, 2019), a screening of the contamination levels of their packaging materials could help food industries to develop a sanitizing process able to achieve sanitization of their packaging devices. This in-depth analysis is of the utmost importance in the light of the fact that the use of UV-C irradiation for industrial sanitizations of food packaging is gaining an increasing interest by food industries, because it is considered a “green” technology and is very cost-effective compared to chemical sanitizers.

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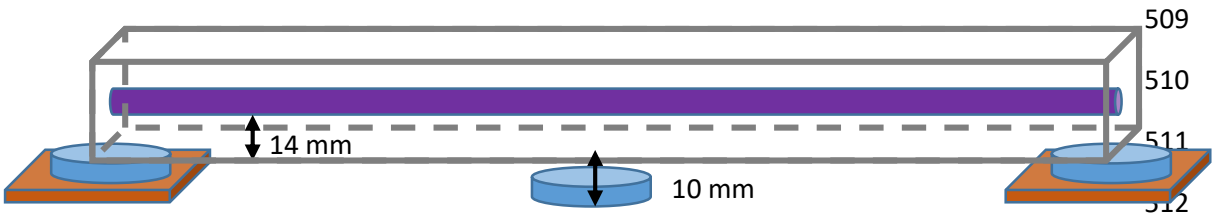
Figure 1 – Multi-layer (A) and single-layer (B) inoculum on the inner surface of HDPE caps. Spatial distribution of the inoculated spores has been drawn based on the pictures captured by a Scanning Electron Microscope (SEM) on dried and metallized spores (data not shown).

Figure 2 – Graphical illustration of the UV-C array with the inoculated cap under the lamp. Yellow dot on the light-blue cap shows the point where spores were inoculated.

Figure 3 - Inactivation curves at 25 °C for each strain (Nh= *Aspergillus hiratsukae,* Em= *Aspergillus montevidense* Tb= *Talaromyces bacillisporus,* Cg= *Chaetomium globosum, Ab*= *Aspergillus brasiliensis*). Graphs describe the effectiveness of UV-C irradiation on single-layer or multi-layer inoculated conidia or ascospores. Vertical error bars indicate standard deviation for mean values (n = 2 for single-layer; n = 3 for multi-layer inoculum trials).

Figure 4 – Logarithmic Reduction Counts (LCR) at 25 °C for each strain at common analysis times ((Nh= *Aspergillus hiratsukae,* Em= *Aspergillus montevidense* Tb= *Talaromyces bacillisporus,* Cg= *Chaetomium globosum, Ab*= *Aspergillus brasiliensis*). For *Aspergillus brasiliensis*, when multi-layer inoculum was considered, values registered at 160 s, 320 s and 480 s were extrapolated from the regression curve. Vertical error bars indicate standard deviation for mean values (n = 2 for single-layer; n = 3 for multi-layer inoculum trials).

Figure 1



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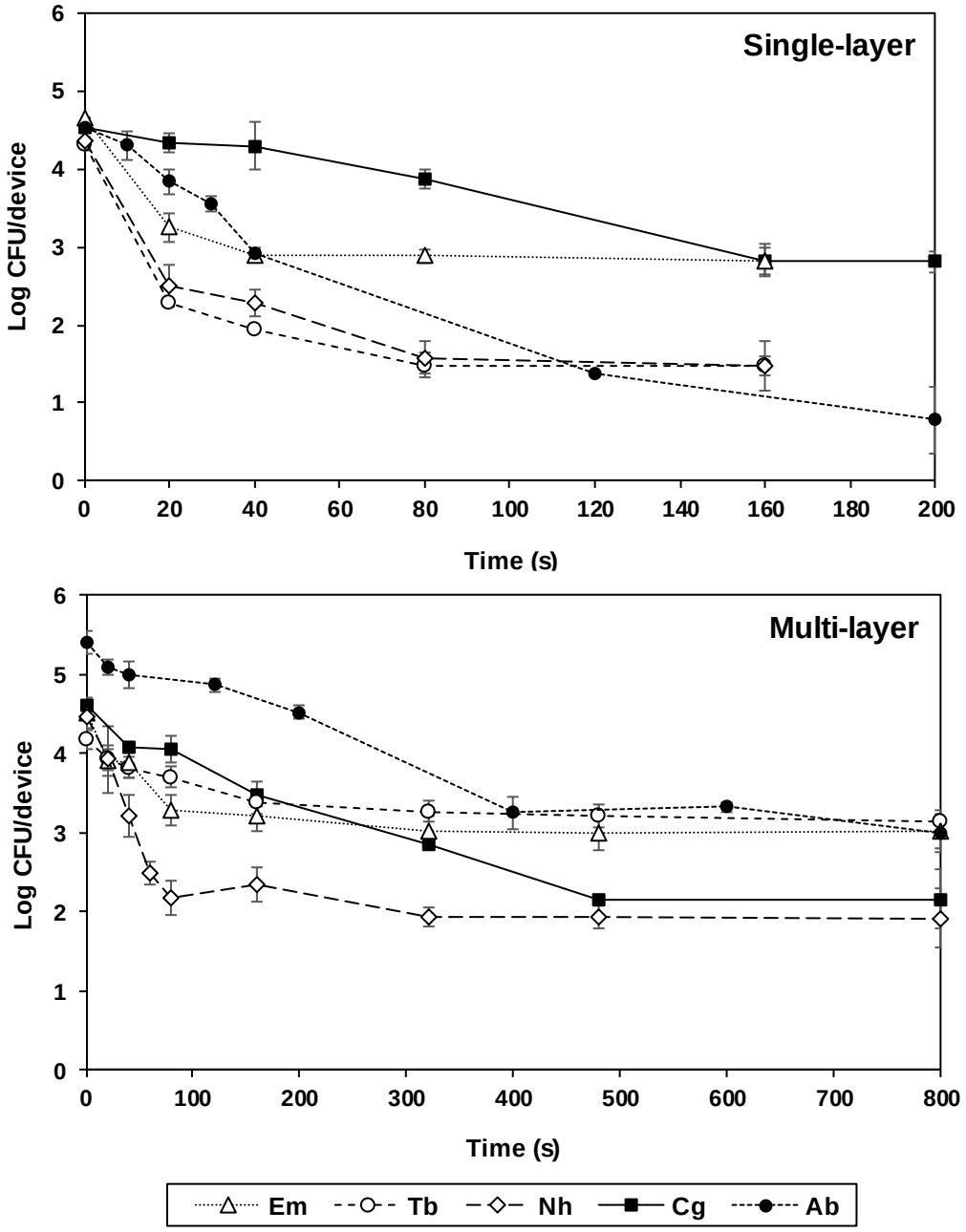
519 Figure 2



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528 Figure 3

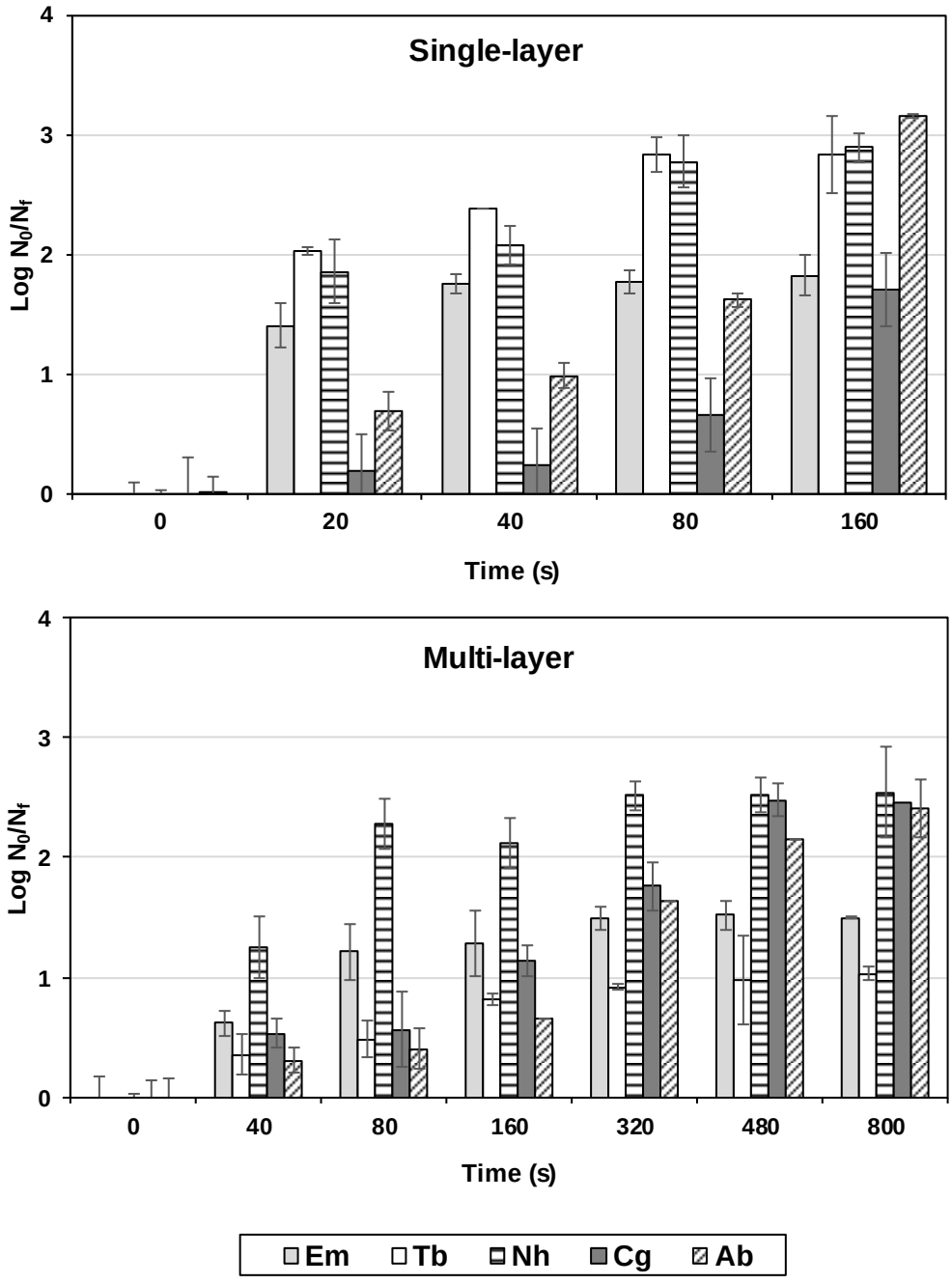

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Table 1 – UV-C resistance of conidia-forming or ascospore-forming strains (Nh= *A. hiratsukae* SSICA 3913, Am= *Aspergillus montevidense* SSICA 28219, Tb= *T. bacillisporus* SSICA 10915, Cg= *C. globosum* ATCC 6205, Ab= *A. brasiliensis* ATCC 16404) inoculated as a single-layer on HDPE caps. Different letters after mean *D* values indicate significant differences ($p \leq 0.05$).

	Log-Linear model[A]					Best fitting models					
Strain	Parameter	Value ± SE	R^2	RMSSE	*1D*-value (s)	Parameter	Value ± SE	R^2	RMSSE	*1D*-value (s)	Reference
Am	Kmax	0.101 ± 0.003	0.89	0.4289	22.8^{c} ± 0.7	Kmax	0.195 ± 0.035	1.00	0.0296	12.2^{c} ± 2.2	B
Tb	Kmax	0.072 ± 0.002	0.72	0.8069	32.2^{bc} ± 0.8	Kmax	0.24 ± 0.02	0.98	0.2620	9.7^{c} ± 0.8	B
Nh	Kmax	0.071 ± 0.002	0.78	0.6747	32.6^{bc} ± 1.1	Kmax	0.185 ± 0.055	0.95	0.4021	13.7^{c} ± 4.1	B
Cg	Kmax	0.025 ± 0.004	0.96	0.1540	96.2^{a} ± 16.7	δ	99.9 ± 16.4	0.99	0.1168	99.9^{a} ± 16.4	C
Ab	Kmax	0.044 ± 0.004	0.92	0.4364	52.9^{b} ± 4.8	Kmax	0.095 ± 0.015	1.00	0.1445	24.9^{c} ± 4	D

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Table 2 – UV-C resistance of conidia-forming or ascospore-forming strains (Nh= *A. hiratsukae* SSICA 3913, Am= *Aspergillus montevidense* SSICA 28219, Tb= *T. bacillisporus* SSICA 10915, Cg= *C. globosum* ATCC 6205, Ab= *A. brasiliensis* ATCC 16404) inoculated as a multi-layer on HDPE caps. Different letters after mean D values indicate significant differences ($p \leq 0.05$).

	Log-Linear model[A]					Best fitting models					
Strain	Parameter	Value ± SE	R^2	RMSSE	*1D*-value (s)	Parameter	Value ± SE	R^2	RMSSE	*1D*-value (s)	Reference
Am	Kmax	0.032 ± 0.003	0.84	0.2109	73.5^{bc} ± 8.5	δ	51.6 ± 4.4	0.98	0.1035	51.6^{c} ± 4.4	D
Tb	Kmax	0.011 ± 0.001	0.87	0.1157	221.7^{a} ± 20.0	δ	147.1 ± 49.8	0.96	0.1023	147.1^{ab} ± 49.8	D
Nh	Kmax	0.069 ± 0.001	0.97	0.1880	33.4^{c} ± 0.5	δ	31.7 ± 4.8	0.97	0.1872	30.3^{c} ± 4.7	
Cg	Kmax	0.011 ± 0.001	0.95	0.2385	211.6^{a} ± 24.5	δ	153.1 ± 55.1	0.95	0.2690	153.2^{ab} ± 55.1	C
Ab	Kmax	0.011 ± 0.000	0.95	0.1740	203.5^{a} ± 8.7	δ	188.2 ± 26.5	0.96	0.2335	188.2^{a} ± 26.5	D

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