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Assessing the potential of ultraviolet irradiation for inactivating waterborne fungal spores: kinetics and photoreactivation studies

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Ultraviolet disinfection has been extensively studied in recent years, especially in bacteria; however, there are still insufficient studies in fungal spores. Moreover, most studies use static batch reactors instead of continuous flow reactors, which are used mainly at the industrial scale. In the present work, the inactivation and reactivation characteristics of two species of filamentous fungi were studied using a single-pass flow-through UV-C reactor (FTR). For this purpose, *Aspergillus niger* and *Penicillium* sp. spores were suspended in water and circulated through the reactor at different UV-C doses. The effects on inactivation and reactivation after 24 in either light or dark conditions were studied. The two fungal strains studied show different UV-C treatment resistance and damage repair capacity. With the experimented FTR system, an inactivation efficiency of up to 2 log units (99% removal) was achieved with doses of 220.1 $\pm$ 24.3 mJ cm^{-2} in the case of *Aspergillus niger* and 123.8 $\pm$ 6.3 mJ cm^{-2} in the case of *Penicillium* sp. The effect of dark repair is negligible, while the photoreactivation process is relevant in the case of *Penicillium* sp., since D_2 increased by 53.8% just after UV-C exposure due to photoreactivation. In general, *A. niger* is more UV-C resistant than *Penicillium* sp.; however, the latter has a greater capacity to photoreactivate.

KEYWORDS

ultraviolet disinfection, fungal spores, photoreactivation, aspergillus, penicillium, flow-through UV-C reactor

1 Introduction

Fungi are a class of heterotrophic eukaryotes, which mainly include molds (also known as filamentous fungi), yeasts (unicellular), and mushrooms (macroscopic fungi) (Mukherjee et al., 2018) and are ubiquitous in the environment (Al-Gabr et al., 2014). The main effect of these fungal spores is related to phytopathogenic diseases (Afonso et al., 2021). In addition, when present in specific environmental matrices, they can produce mycotoxins showing toxicities to invertebrates, plants, and microorganisms (Doggett, 2000). In recent years, the occurrence of filamentous fungi in the aquatic environment has been of growing interest because they can cause odor, taste, and turbidity problems in the water (Wan et al., 2023).

Furthermore, studies report that once the fungi sprout rapidly, they would cause serious safety problems in the drinking water supply, causing asthma, allergic pneumonia, skin infection, and other diseases in people with low immunity (Al-Gabr et al., 2014). Several studies have reported the presence of filamentous fungi in drinking water distribution systems worldwide (Afonso et al., 2021). A first study of this type reported that fungi existed in systems of urban water supply biofilms, and the number of filamentous fungi ranged from 4.0 to 25.2 CFU cm^{-2}, of which Aspergillus and Penicillium were the dominant genera (Doggett, 2000). In addition, *Aspergillus*, *Trichoderma*, *Fusarium*, *Mucor*, and *Rhizopus* were dominant in surface systems (Hageskal et al., 2007). And locally, a study demonstrated the presence of filamentous fungi in the rivers of the city of Cuenca-Ecuador (Carlos Rivera y Liliana Ochoa, 2018). In addition, fungi are not considered in international water quality guidelines and are not monitored in drinking water. This may be partly due to the low importance given to this issue in drinking water management, as well as technical situations related to the difficulty of the water supplier to identify fungal species and the consequent lack of knowledge of the variability of the species present in the water. What also contradicts; the mission established in the Sustainable Development Goal (SDG) 6 states: "Ensure availability of water and its sustainable management and sanitation for all" (UNICEF and WHO, 2017).

Disinfection of waterborne fungi is essential (Wan et al., 2023). Unfortunately, conventional drinking water treatment processes (coagulation-sedimentation-filtration) cannot remove fungi from water (Al-Gabr et al., 2014). Therefore, it will be necessary to explore further efficient, safe, and economical disinfection technologies for fungal inactivation in future studies. Under this context, studies have shown that applying ultraviolet (UV-C) radiation efficiently inactivates fungal spores (Wan et al., 2022; 2023; Wu et al., 2022). UV-C irradiation, with wavelengths between 200 and 320 nm, has been considered an effective disinfection technology in recent years (Hijnen et al., 2006; Kowalski and Kowalski, 2009). It is, therefore, widely used in water and wastewater treatment due to its versatility, efficiency, and low potential for forming harmful disinfection by-products (Song et al., 2016). The most commonly used UV-C sources are the low-pressure monochromatic emission (LPUV) with a wavelength of 254 nm and the medium-pressure mercury lamp (MPUV), which emits polychromatic light in a wide range of wavelengths, between 200 and 400 nm (Ao et al., 2018).

At the research-experimental level, UV-C disinfection can be applied using various devices, the most widely used being the collimated beam reactor (CBR). Studies have mainly focused on analyzing the application of UV-C radiation using static CBRs because it allows the maximum precision on the intensity calculation using a radiometer and direct control of the exposure time (Al-Gabr et al., 2014; Wen et al., 2017; Wan et al., 2020; Xu et al., 2022). However, their operation differs significantly from commercial UV-C disinfection systems, which commonly use flow-through reactors (FTRs). In this context, CBR often used as a reference and calibration for other more complex disinfection equipment (Bolton, 2000), while continuous FTRs are more similar to commercial UV-C systems (Lindenauer and Darby, 1994) and are therefore more focused on the application of full-scale treatments.

The inactivation of microorganisms by UV-C radiation emission is explained by the fact that it affects the formation of cyclobutane pyrimidine dimers (CPD) and six to four pyrimidine dimers in the DNA strands of microbial cells after irradiation, so the existence of these UV-C-induced lesions would prevent standard DNA transcription and replication, leading to the inactivation of microorganisms (Setlow and Carrier, 1964). However, microorganisms inactivated by UV-C light have a high risk of reactivation by means of two main processes, photoreactivation and dark repair (Lindenauer and Darby, 1994), thus significantly weakening the efficiency of disinfection. Photoreactivation is a conventional repair system widely present in organisms in which the enzyme photolyase is activated by photon energy with wavelengths from 330 to 480 nm and binds specifically to cyclobutane-pyrimidine dimers and reverses the damage (Song et al., 2019). On the other hand, dark repair is a light-independent process that replaces damaged DNA with new and intact nucleotides (Sinha and Häder, 2002). Among both methods, photoreactivation is the main one in terms of the damage that can be repaired (Nebot Sanz et al., 2007).

The present study aimed to explore the inactivation and reactivation characteristics of *Aspergillus niger* and *Penicillium* sp. spores present in water after being irradiated by UV-C at different doses through a continuous flow laboratory reactor. Viable fungal spores were measured immediately after UV-C irradiation and after a 24-h incubation period in an illuminated or dark environment. Inactivation curves were fitted to inactivation models to determine kinetic parameters that allowed quantitative comparison of UV-C-resistance and damage repair potential of the two fungal strains and post-treatment conditions tested.

2 Materials and methods

2.1 Organisms and microbiological procedures

This study used two strains of the fungal species *A. niger* and *Penicillium* sp., acquired from the Fungi Collection of the University of Azuay (Ecuador). These strains were chosen as model microbes because they are the dominant genera of fungal spores isolated from water sources; in addition, they are representative genera of water contamination.

The biological procedures were based on previous studies (Pereira et al., 2013; Wen et al., 2017; Wan et al., 2022). First, the strains delivered were reactivated in 30 mL of potato dextrose agar (PDA) culture medium at 27°C for 2 weeks. They were then inoculated on dichloran rose bengal chloramphenicol agar (DRBC) and cultured in an incubator at 27°C for 2 weeks. Subsequently, the grown spores were scraped from DRBC agar and washed three times with sterile phosphate buffer solution (PBS) at pH = 7.3 by centrifugation (8000 rpm, 10 min).

The spores concentration was determined using a Neubauer chamber. Then a volume of distilled with a phosphate-buffer solution water was added, aiming to obtain a concentration

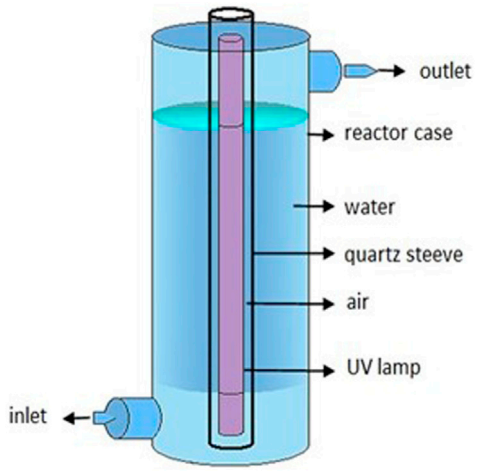

FIGURE 1
Elements of the low-pressure mercury UV-C lamp in the experiment.

TABLE 1 UV-C lamp characteristics of the experimental system.

Part	Parameter and abbreviation		Value
UV-C lamp	Total power	P	6 W
	Power at 254 nm	P_{254}	2 W
	Length	L	18.9 cm
Quartz sleeve	Outer radius	r_Q	1.2 cm
	Thickness	e	1.6 mm
	Transmitance	T_Q	0.94
Reactor case	Inner radius	r_R	2.55 cm

between 10^7—10^8 spores mL^{-1}. This stock solution was stored under refrigeration (4°C) until the time of experimentation.

The surface spreading and plate counting technique was used to quantify the concentration of viable microorganisms after the experiments (Hoben and Somasegaran, 1982). Plates with a concentration between 20 and 300 spores were selected as representatives. To achieve this concentration, serial dilutions of each sample were made at a ratio of 1:10.100 µL of each dilution was then taken and evenly dispersed in 90 mm Petri dishes with DRBC agar. These plates were incubated at 27°C for 3 days. This culture time was left, due to the space they begin to occupy once they start to develop, determining that this is sufficient time to obtain adequate development and an accurate estimate of the number of spores in the sample. After incubation, the spore concentration was counted and expressed as spores mL^{-1}.

The UV-C treatment was applied by means of a single-pass through a ring-type continuous flow-through reactor, built at laboratory scale. The reactor was equipped with a low-pressure (LP) mercury vapor lamp (Phillips 1GPM - in/out 1/4″monochromatic emission at 254 nm) covered with a quartz sleeve and inserted in an aluminum housing (Figure 1; Table 1). The reactor was inserted in a laboratory rig composed of a 20 L plastic tank, a centrifugal pump, manual valves to manipulate the flow rate and, finally, the UV-C reactor.

The UV-C dose applied (Eq. 1; abbreviations and values in Table 1) was determined according to USEPA specifications (USEPA, 1986), as the product of the mean intensity (I_m) and the theoretical retention time (TRT). The power output at 254 nm (P_{254}) was calculated as one-third of the total lamp power (Figawa, 2009). The TRT was calculated as the quotient between the volume of water exposed to UV-C light (V_R) and the flow rate (Q). I_m was calculated according to a geometrical model of radiation scattering for ring reactors (Eq. 2), validated by biodosimetry in previous studies (Romero-Martínez et al., 2014; Moreno-Andrés et al., 2017). Water transmittance at 254 nm (T_W) was measured at the beginning of each test using a Genesys 20 spectrophotometer, providing values between 93.5% ± 0.5%. Depending on the value of T_W, I_m 8.73 ± 0.34 mW cm^{-2}.

$$D = TRT \cdot I_m \tag{1}$$

$$D = \frac{V_R}{Q} \cdot \frac{P_{254}\, T_Q^e}{2\, L\, \pi^2 \left(r_R^2 - r_Q^2\right)} \iint_{r_Q}^{r_R} \frac{T_W^{r-r_Q}}{r} \cdot \mathrm{dx\,dy} \tag{2}$$

2.2 Experimental procedure

The stock solution containing the fungal spores was placed in the 20 L plastic tank containing distilled buffered water, obtaining a concentration between 10^5 and 10^6 mL^{-1}, and then acclimatized for 30 min, keeping it shaken. The UV-C lamp was connected 5 min before the experiment to reach a stable radiation emission (Bolton, 2000). The pump was then turned on to allow the water to pass through the UV-C reactor. The flow rate, required to calculate the TRT, was measured using a 1 L graduated cylinder and a stopwatch. Once the flow rate stabilized and measured, a volume greater than the capacity of the hydraulic system was wasted prior to the sample collection, ensuring that the sample came directly from the tank and received the calculated dose. The sample was collected in a sterile 250 mL borosilicate bottle at the reactor outlet. To avoid contamination of the section downstream of the reactor, the UV-C doses were applied in descending order, that is, starting from the lowest flow rate. After collecting a sample, the flow rate was increased and the process was repeated again. Samples were collected at flow rates between 21 and 400 L h^{-1}, corresponding to TRT between 51.4 and 2.7 s and UV-C doses of 179.2 and 23.04 mJ cm^{-2}, respectively. The control for each experimental series was collected by pumping the target water at the maximum flow rate after turning off the UV-C lamp; this allows for excluding any possible mechanical stress from the inactivation caused by the UV-C irradiation.

The sample volume was divided into three 20 mL test tubes. One of these tubes underwent immediate plate counting on the same day of the test to determine the number of surviving spores after UV-C inactivation (0 d). Another tube was covered with aluminum foil to replicate dark conditions (1 d—dark), while the remaining tube was left uncovered to simulate light conditions (1 d—light). These two tubes were incubated in a shaking incubator (model FS-70B) at 20°C for 24 h. The incubator was equipped with a fluorescent lamp emitting 36 µEinstein m^{-2} s^{-1} of light emitted at a wavelength

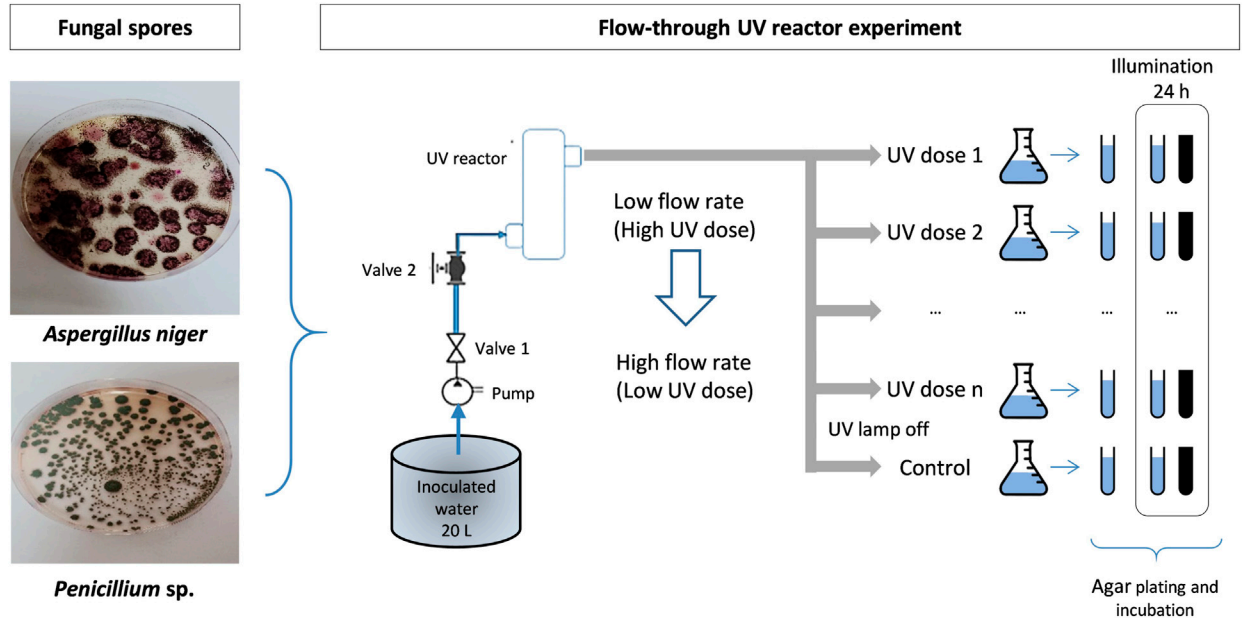

FIGURE 2
Scheme of the laboratory plant and experimental procedure.

between 450 and 500 nm. The experimental procedure is outlined in Figure 2.

2.3 Determination of the disinfection kinetics parameters and statistical analyses

Survival (S) in each sample was determined as the quotient of the culturable spore concentration in the irradiated sample between the spore concentration in control without irradiation and without being subjected to post-treatment incubation. Inactivation curves were obtained by plotting Log(S) *versus* the UV-C dose applied in each case. For determining the existence of significant differences between the UV-C treatment for the different organisms under the different post-treatment conditions, the inactivation curves were subjected to ANCOVA analysis with Statgraphics Centurion (version.16.1.03), using Log(S) as the dependent variable, UV-C dose as covariable, and the target organism (*A. niger* or *Penicillium* sp.) and the post-treatment (0 days, 1 day in illuminated conditions or 1 day in dark conditions) as factors. The inactivation kinetics parameters were obtained by modeling the inactivation curves with the GInaFiT tool for MS Excel (Geeraerd et al., 2005). These parameters were used to calculate the dose required to reduce the initial concentration by two orders of magnitude (D_2), that is, the inactivation of the 99% of the target organisms, as an estimator of treatment efficacy.

To evaluate the effect of photoreactivation, the photoreactivation percentage (*PPR*) was calculated according to Eq. 3 (Lindenauer and Darby, 1994), where: N_p = bacterial concentration of the photoreactivated sample (CFU mL^{-1}), N = bacterial concentration immediately after UV-C disinfection (CFU mL^{-1}), N_0 = bacterial concentration before UV-C disinfection (CFU mL^{-1}). This concept quantifies the percentage of photoactivated bacteria among the bacteria affected by UV-C irradiation.

$$PPR\ (\%) = \frac{N_p - N}{N_0 - N} \cdot 100 \quad (3)$$

3 Results and discussion

3.1 Analysis of the inactivation curves

The inactivation curves obtained for *A. niger* and *Penicillium* sp. (Figure 3) showed a dependence between the Log(S) and the applied UV-C dose. The ANCOVA analysis indicated that the organism ($p < 0.001$) and the UV-C dose applied ($p < 0.001$) influence significantly the UV-C treatment outcome. In the case of *A. niger*, although the inactivation curves indicate a slightly lower efficacy when the treated organisms were incubated under light for 24 h, the ANCOVA analysis indicated no significant differences between the three post-treatment conditions ($p = 0.683$). On the other hand, there were significant differences between the post-treatment conditions in the case of *Penicillium* sp. ($p < 0.001$), determining one homogeneous group with both 0 days and 1 day under dark conditions and another homogeneous group with 1 day under illuminated conditions. Therefore, according to the inactivation curves analysis, the spores of *A. niger* are more UV-C-resistant with respect to the *Penicillium* sp. spores. The photoreactivation is significant in the case of *Penicillium* sp. These facts have been evaluated quantitatively by determining the inactivation kinetics and the percentage of photoreactivation.

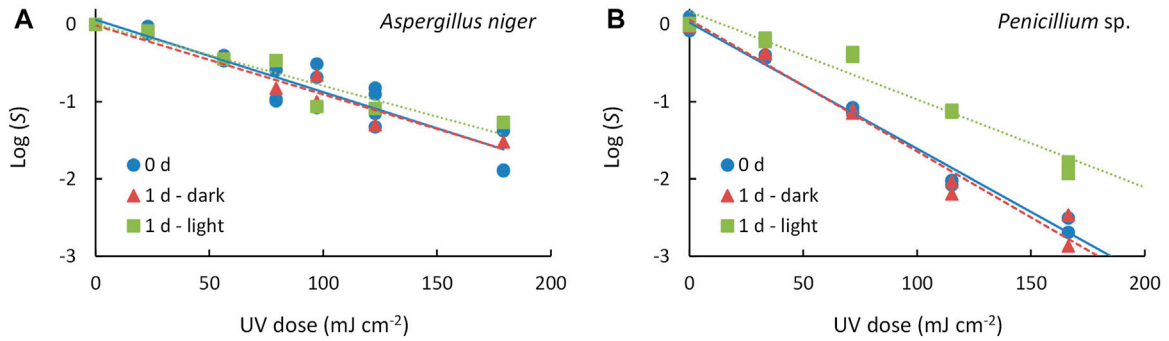

FIGURE 3
Inactivation curves of **(A)** *Aspergillus niger* and **(B)** *Penicillium* sp. for different post-treatment conditions: absence of post-treatment incubation (0 d), one-day incubation in dark environment (1 d—dark) and one-day incubation in illuminated environment (1 d—light). Symbols represent the experimental data and lines represent the inactivation model fitted (solid line: 0 d, dashed line: 1 d—dark, dotted line: 1 d—light).

TABLE 2 Estimated kinetic values for the two strains studied in different post-treatment conditions and statistical values of the fit to the models.

Organism	Post-treatment	Log (S_0)	k (cm^2 mJ^{-1})	R^2	D_2 (mJ cm^{-2})
A. niger	0 d	0.06 ± 0.11	0.022 ± 0.002	0.836	220.1 ± 24.3
	1 d—dark	−0.01 ± 0.12	0.021 ± 0.003	0.921	222.2 ± 31.5
	1 d—light	0.00 ± 0.12	0.018 ± 0.003	0.900	251.4 ± 38.5
Penicillium sp.	0 d	0.02 ± 0.06	0.038 ± 0.002	0.986	123.8 ± 6.3
	1 d—dark	0.06 ± 0.09	0.039 ± 0.002	0.975	120.9 ± 8.5
	1 d—light	0.17 ± 0.09	0.026 ± 0.002	0.949	190.4 ± 16.7

3.2 UV-C treatment inactivation kinetics

First-order kinetics was observed between the S and the UV-C dose applied up to 180 mJ cm^{-2} (Eq. 4), with high level of correlation, according to the values of R^2 (Table 2). In absence of post-treatment incubation, the inactivation constant (k) was 0.022 cm^2 mJ^{-1} for *A. niger* and 0.038 cm^2 mJ^{-1} for *Penicillium* sp. The Log(S_0) values of the incubated samples represent the effect of incubation on the survival of the organisms without considering the impact of UV-C treatment. The values in all cases are close to 0, indicating that incubation alone does not generally affect survival. According to the log-linear model parameters, the UV-C dose required to achieve 2 log-reductions (D_2) was 220.1 ± 24.3 mJ cm^{-2} in the case of *A. niger* and 123.8 ± 6.3 mJ cm^{-2} for *Penicillium* sp.

$$S = S_0\, e^{-k\,D} \tag{4}$$

In the present study, no tails were present for the UV-C range experimented in the three treatments (inactivation and reactivation in light and dark) (Figure 3), nor were shoulders observed. Comparing with previous studies (Wen et al., 2017; Wan et al., 2020) experimented with these two genera in a collimated reactor with 254 nm low-pressure mercury lamps and found tailing and shoulder phenomena; they concluded that this phenomenon is present regardless of incubation time and emission wavelength. Therefore, we also support the hypothesis that these phenomena are probably due to spore aggregation (Mattle and Kohn, 2012), hydraulic problems in treatment with recirculation and/or screening by suspended solids and flocs, as well as achieving inactivation levels close to the detection limit (Azimi et al., 2017; Carré et al., 2018; Romero-Martínez et al., 2019). In this study, the non-significant values of S_0 indicate absence of shoulder and thus, suitability of the log-linear model for fitting the obtained data. On the other hand, tailing cannot be discarded at UV-C dose beyond the experimental range of UV-C doses applied.

The results obtained revealed significant differences in UV-C resistance between the two strains. Comparing the inactivation profiles (d 0), *Penicillium* sp. showed higher sensitivity to UV-C light in comparison with *A. niger*. This fact was mainly attributed to differences in enzymes, intracellular structure diversity and pigment content within the fungal cells (Guo et al., 2011; Liu et al., 2014). The genus *Aspergillus* has been reported to have as its main defense mechanism the production of melanins and other pigments (Clauß, 2006; Wu et al., 2022), which makes it more tolerant compared to other species studied (Narita et al., 2020). In the case of *Penicillium* sp., carotenoids play a protective role against UV-C exposure (Guo et al., 2011). However, this defense system is weaker when compared to that of the genus *Aspergillus*. Moreover, these defense mechanisms are more significant, especially in wild-type species *versus* laboratory strains (Esbelin et al., 2013). Betzalel et al. (2020) reported that spores of fungi such as *Penicillium*, *Aspergillus* and *Acremonium* are hydrophobic and tend to cluster together, which

makes them resistant to UV-C inactivation. Nourmoradi et al. (2012) investigated the effects of UV-C irradiation on *Aspergillus* sp. and found that a dose of 20.7 mJ cm^{-2} was necessary to achieve a 4-log reduction.

Comparing the reactor configuration, studies (Wen et al., 2019) found that *k* for *A. niger* was relatively lower than for *Penicillium polonicum*; the results of this study agree with this fact. However, the reported values are considerably higher (0.0638–0.0368 cm^2 mJ^{-1}) than those found in this study. It will be necessary to know whether the reported inactivation efficiency is maintained or decreased when experimenting under continuous flow reactor conditions, also considering reactivation. Generally, in batch reactors such as the collimated beam reactor, a higher dose is required to achieve disinfection levels similar to the continuous reactor (Vélez-Colmenares et al., 2011; Silva et al., 2013). This is explained by the fact that collimated reactors have a low intensity, and a longer exposure time is required to achieve the same dose than with a continuous flow reactor; on the contrary, those can be constructed to emit radiation with a higher intensity and therefore require a shorter exposure time. Therefore, experimenting in one or the other reactor will cause a different effect on survival and subsequent reactivation (Romero-Martínez et al., 2019). In addition, this fact is attributed to variables such as the heterogeneous distribution of UV-C irradiance in the exposed volume and the photorepair processes of damage, which become more relevant with increasing exposure times (Harm, 1980). These facts imply the necessity of using FTRs in inactivation experiments instead static batch operating CBRs.

It was found that a higher UV-C dose was required to achieve fungal spore reduction, compared to other microorganisms; for example, typical indicator organisms such as *Escherichia coli*, in a study performed with the same reactor type, it was found that *E. coli* ATCC 8739, required a dose of 15 mJ cm^{-2} for a reduction of 5 log10 (Duque et al., 2022), being this even one of the bacterial strains that have shown greater resistance to UV-C treatment (Romero-Martínez et al., 2023). The inactivation rate constants (*k*) found (0.022–0.039 cm^2 mJ^{-1}) were significantly lower than those reported for other types of microorganisms; *E.coli* (0.35–0.91 cm^2 mJ^{-1}), *Pseudomonas aeruginosa* (0.51–0.74 cm^2 mJ^{-1}), *Legionella pneumophila* (0.45–0.86 cm^2 mJ^{-1}) and other bacteria (Beck et al., 2017; Zhou et al., 2017; Nyangaresi et al., 2018; Rattanakul and Oguma, 2018; Romero-Martínez et al., 2023), even for some viruses such as coliphage MS2 (0.061–0 cm^2 mJ^{-1}) (Mamane-Gravetz et al., 2005) and bacteriophage Qβ (0.056–0.098 cm^2 mJ^{-1}) (Ben Said et al., 2010; Li et al., 2019; Keshavarzfathy et al., 2021), accounting for the superior UV-C-resistance by the studies fungi spores in comparison with other microorganisms, which is attributed to the structural differences between fungi, bacteria and viruses, as well as the increased UV-C-resistance by the spore cell wall, composed of chitin, making fungi more UV-C-resistant (Wan et al., 2023).

3.3 Effect of the post-treatment incubation on the treatment efficacy

The inactivation curves analysis indicated that the dark repair after UV-C irradiation was not significant, and the photoreactivation was significant in the case of *Penicillium sp.*

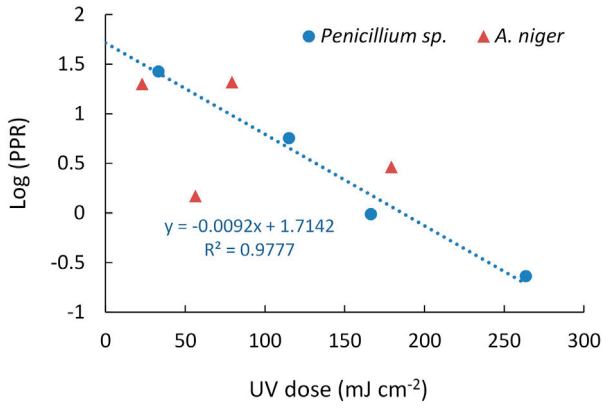

FIGURE 4
Relationship between the Log(*PPR*) and UV-C dose for *Aspergillus niger* and *Penicillium* sp.

This implies that the values of D_2 (Table 2) obtained for samples incubated for 24 h in dark conditions were similar to those obtained without incubation, indicating that the inactivation achieved by the UV-C treatment is maintained for at least 1 day is the irradiated spores are kept in a dark environment. On the other hand, the *Penicillium* sp. samples incubated for 24 h in illuminated conditions showed a D_2 value that increased a 53.8% with respect to the D_2 right after the UV-C exposure, due to the photoreactivation.

The percentage of photoreactivation (*PPR*) was determined for both organisms and correlated with the UV-C dose according to a first order kinetics (Log(*PPR*) = a + b · UV-C dose) (Figure 4). In the case of *A. niger*, the *PPR* ranged between the 1.5% and 20%; the regression analysis reported not significant linear correlation between Log(PPR) and the UV-C dose applied (p = 0.572). On the other hand, *PPR* ranged between the 0.2 and the 30% for *Penicillium* sp., with a significant linear correlation between Log(*PPR*) and the UV-C dose (p = 0.011) (Figure 4). According to the regression parameters determined for *Penicillium* sp., a 13.7% of the spores can be photoreactivated after 1-log reduction, a 3.7% after 2-log reductions and a 1.0% after 3-log reductions. Therefore, treating the *Penicillium* sp. spores with higher UV-C doses increases the inactivation level as well as reduces the potential photoreactivation.

The process of repair involves DNA damage reversal mechanisms, such as photoreactivation and dark repair. In this study, it was found that using a low-pressure mercury lamp for UV-C treatment with emission of 254 nm, in a continuous flow reactor, did not completely prevent fungal strains from repairing some of the damage if they were exposed to environmental light after treatment. Previous studies (Wen et al., 2017; Wu et al., 2022; Xu et al., 2022) have highlighted the importance of photoreactivation in the inactivation process. In this study, irradiated samples were exposed to light in a culture chamber to enhance photorepair, the percentage of photoreactivation varied among fungal spores at different UV-C doses ranging from 23 to 260 mJ cm^{-2}, *Penicillium* sp. was able to achieve the highest percentage of photoreactivation, up to 27%, at a dose of 33.27 mJ cm^{-2}. These results are consistent with previous research (Wen et al., 2019), which demonstrated that

Penicillium sp. exhibited the most substantial photoreactivation, while *A. niger* showed low levels of photoreactivation. Finally, a study by Romero-Martínez et al. (2023) compared photoreactivation in several types of bacterial strains at 24 h and found a maximum of 48% for *E. faecalis* ATCC 27285. These results suggest that fungal spores typically have a lower maximum percentage of photoreactivation compared to bacteria. For example, Wen et al. (2019) reported a photoreactivation percentage of 27% for *Penicillium* sp., which was lower than that of *Escherichia coli* (72.83%) under similar conditions. Our study presents unique results on the inactivation of two fungal strains using a continuous FTR, which has not been previously reported.

The rate of photoreactivation is influenced by the storage conditions of the irradiated water (Bohrerova and Linden, 2007; Maghsoodi et al., 2022). For instance, if the water is stored in sunlight, there is a trade-off between photoreactivation and subsequent inactivation caused by UV-C-A and UV-C-B radiation (Lindenauer and Darby, 1994; Kashimada et al., 1996; Tosa and Hirata, 1999; Salcedo et al., 2007; Marugán et al., 2008; Quek and Hu, 2008; Rubio et al., 2013; Rodríguez-Chueca et al., 2015). During the reactivation process, a fluorescent lamp was used whose emission range includes the optimal wavelengths for photolyase activation, from 350 to 500 nm (Xu et al., 2022). In the case of *Penicillium* sp, it was observed to show signs of successful reactivation. In this sense, it can be assumed that the light used in incubation is effective for photoreactivation, and thus, the lack of photoreactivation in *A. niger* may be due to the specific characteristics of that organism, which make it less prone to be photoreactivated.

The results obtained in this study indicated the absence of dark repair within 24 h after UV-C irradiation, which is consistent with previous studies (Wan et al., 2020). Although not significant, this can influence the photoreactivation processes if conjugated during the application of UV-C radiation; it is known that a 3 h delay in light exposure after UV-C irradiation significantly reduces the photoreactivation fractions (Hallmich and Gehr, 2010). Therefore, this dark delay can be introduced as part of water treatment to prevent risks due to photoreactivation. However, this is validated for bacteria; it is unknown if the same will be valid for fungal spores. These results support the need to consider the post-treatment management of the water once treated with UV-C; since higher doses of UV-C will be required to maintain, for example, the same goal of 3 log reductions if the treated water is exposed to light conditions after treatment (Figure 2), or else consider the possibility of keeping the water in darkness after treatment and before use. It is therefore proposed that future studies are needed to determine the hours of dark storage necessary for the fungal strains not to reactivate, or to reactivate a minimum target percentage, once exposed to light.

3.4 Implications of the UV-C inactivation and reactivation mechanisms for the abatement of fungal spores in water

It is of interest to study the mechanisms of UV-C inactivation and reactivation in different microorganisms and environmental conditions to ensure the effectiveness of disinfection and to avoid the selection of resistant strains. The implication of these mechanisms in reducing fungal spores in water is significant since water treatment systems must consider not only microbial inactivation but also the possibility of reactivation. Exposure of fungal spores to UV-C radiation may not be sufficient to completely eliminate them if adequate light conditions are present for photoreactivation. Thus, the importance of taking into account storage conditions after UV-C treatment is directly related to the photoreactivation ability of fungal spores. As previous research has shown, some types of fungal spores may be more resistant to UV-C radiation and have a higher photoreactivation capacity than others (Wan et al., 2020; 2022). In the case of *Penicillium* sp. it is requires less UV-C dose. However, it has a higher photoreactivation capacity, but even considering photoreactivation, it requires less UV-C dose than *A. niger*. This suggests that in the treatment of water contaminated with *Penicillium* sp. spores, the storage conditions after treatment will not significantly impact the treatment's effectiveness. On the other hand, in the case of *A. niger*, storage conditions after treatment may be more important. In addition, these studies are important for optimizing the design and scale-up of UV-C radiation reactors at the commercial level, considering the different inactivation and photoreactivation conditions in fungal spores and storage conditions.

Spore inactivation is a key factor in ensuring the microbiological safety of water and other products, especially in the food and pharmaceutical industry. However, photoreactivation can decrease the effectiveness of treatment, which can increase the risk of microbiological contamination. Therefore, it is important to consider the specific characteristics of each fungal spore species when designing water treatment systems and other products.

Based on the findings of this study, it is essential to conduct further research to examine the resistance and repair capacity of diverse fungal strains in response to UV-C treatment. Additionally, conducting targeted experiments to explore the photoreactivation process in Penicillium sp. is recommended. This experimentation should encompass varying wavelengths and exposure durations to determine the optimal effects. Lastly, assessing the impact of environmental factors beyond light, such as temperature and the presence of chemical compounds, is crucial on the fungi's photoreactivation ability. These recommendations will contribute to a more comprehensive understanding of the repair and photoreactivation mechanisms, ultimately enhancing the efficacy of UV-C treatment.

4 Conclusion

The results show that the two strains of fungi studied, *A. niger* and *Penicillium* sp. offer different resistance to UV-C treatment and capacity for damage repair. The effect of repair in darkness is insignificant, while the photoreactivation process is relevantin the case *Penicillium* sp. In general, *A. niger* is more resistant than *Penicillium* sp.; however, the latter has a greater capacity for photoreactivation.

The FTR is effective in inactivating fungal spores in water. It was demonstrated that inactivation could be up to 2 log-reductions with UV-C doses of 251.4 ± 38.5 and 190.4 ± 16.7 mJ cm^{-2} and taking into account photoreactivation processes for 24 h, for *A. niger* and *Penicillium* sp. respectively.

The use of FTR demonstrates that it could be a valuable tool for the investigation and optimization of fungal spore inactivation at the laboratory level. This type of reactor allows a better reproduction of the processes at full scale, resulting in a more accurate extrapolation of the results obtained in the laboratory. In addition, the more precise control of process parameters in a continuous flow reactor can improve the efficiency and reproducibility of the results, which is crucial for the research and development of microorganism inactivation technologies in practical applications.

Finally, the method used, including the photoreactivation procedure, can be used to evaluate the microbiological quality of UV-C-disinfected water for filamentous fungal strains. The increased UV-C dose should be considered during the design of UV-C disinfection when UV-C-disinfected water is discharged into open receiving systems, such as rivers or lakes, where they are exposed to light conditions, which may favor photoreactivation processes.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://estliveupsedu-my.sharepoint.com/:x:/g/personal/pduque_ups_edu_ec/EfNtGcPEMrZGvgWTDcRahMQBj9bJhB-sC76Ld_9pDlV30w?e=0KgYoS

Author contributions

PD-S writing—original draft, writing-review and editing, methodology, investigation, data curation, conceptualization, project administration, funding acquisition. ND-A methodology, investigation, conceptualization. LR-M writing—review and editing, conceptualization, supervision. VP-V review, editing, supervision, formal analysis. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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