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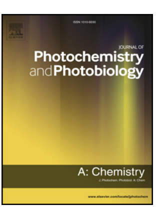
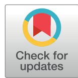


Photocatalytic paint for fungi growth control under different environmental conditions and irradiation sources



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ABSTRACT

The fungicide effect employing *Aspergillus niger* as a representative microorganism was tested applying a Photocatalytic paint formulated with an anatase carbon doped TiO_2 and compared with a homemade non-active paint (rutile TiO_2), a commercial normal indoor latex paint and a commercial “antifungal” latex paint. For this purpose *Aspergillus niger* conidia inactivation on the paints coatings was performed under visible and UV radiation applying different experimental conditions (relative humidity, radiation flux and absence of light).

Under visible light no significant difference in conidia inactivation was found for all studied paints. Nevertheless, the Photocatalytic paint under UV radiation presented around 1.4 times higher fungus inactivation than the blank control paints. In order to evaluate the damage produced by visible or UV radiation over the conidia and to analyze the ability of repairing it, incubation at optimal germination conditions of Photocatalytic and normal paints after irradiation treatment was performed. For conidia irradiated on Photocatalytic paint, not only conidia could not germinate but also the inactivation continued even after the end of the irradiation assay. Finally, *A. niger* conidia was exposed over a culture medium with or without the paints pigments (Carbon doped TiO_2 or Rutile) under different types of radiation (visible and UV). Much greater growth control of vegetative forms was obtained by applying photocatalytic TiO_2 under both types of light.

It can be concluded that the developed Photocatalytic paint presents a higher ability to control fungal growth compared to the other paints studied and could be applied for indoor air decontamination. Also, it was possible to control the dissemination agent that can generate more mold growth on surfaces and affect the people health.

1. Introduction

Diverse microorganisms are transported as bioaerosols, being this a health risk for people living in indoor environments. A particular but very common problem is the fungus or mold growth in environments without ventilation and with high relative humidity [1]. The exposure to these microorganisms is associated to the development of asthma and allergy, among other respiratory problems [2]. Additionally, the fungus development on walls can be a deterioration cause of these surfaces. Nowadays, there are different wall paints or paint additives with antifungal properties employing chemical fungicides. However, some of these antifungal compounds at certain concentrations are dangerous for humans and the environment [3].

The heterogeneous photocatalysis is an efficient method for the chemical and biological purification of water and air. The antimicrobial power of this technology has been widely studied employing UV radiation, but less investigated applying visible light as the energy source [4–8]. In these works the inactivation of virus, bacteria and fungus was assessed. One of the model environmental and non-pathogen molds employed in photocatalytic works is *Aspergillus niger* principally under UV-A and UV-C radiation [5,7–17].

An increasingly common application of heterogeneous photocatalysis is the combination of TiO_2 with different building materials [18–20] which gives self-cleaning and air-purification properties. In previous works in our group, a photocatalytic paint containing a commercial carbon doped TiO_2 with an extended photoactivity in the visible light spectrum was formulated. This paint was tested for a frequent volatile organic compound of indoor air, acetaldehyde, observing satisfactory results when the samples were irradiated with fluorescent visible light lamps [21].

The antimicrobial properties of photocatalytic materials, like functional wall paints, have shown to possess potential and attractive applications in everyday life [22]. However, the environmental fungi control applying paints containing anatase TiO_2 was principally tested employing UV radiation observing the growth inhibition of the vegetative form of *Aspergillus niger* on the photocatalytic surface [23]. On

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the other hand, in order to evaluate the inhibition of growth using also interior lighting, the diameters of vegetative colonies of different environmental molds were measured after different irradiation times in a culture medium covered with different photocatalytic paintings [24]. While some tested photocatalytic paints could inhibit the growth of some environmental fungus, for *Aspergillus niger* the inhibition on the photocatalytic surfaces irradiated with visible light was similar to the results found on culture medium alone.

In the present work, the environmental fungus growth control was evaluated employing the developed photocatalytic paint (Antatase carbon doped TiO_2), a homemade non-active paint (Rutile TiO_2), a commercial normal indoor latex paint, and a commercial antifungal latex paint. Different types of illumination (UV-A and visible light), relative humidity and radiation flux were applied for the inactivation of *Aspergillus niger* conidia, the selected model microorganism, on the different paints coatings. Therefore, the reduction of the *Aspergillus niger* dispersion propagule that could disseminate more mold colonies on surfaces was assessed. The obtained experimental results were theoretically and statistically analyzed through the exponential regression of Colony Forming Units (CFU) and applying an experimental design and a factorial analysis of variance. Additionally, the conidia damage caused by the photocatalytic treatment was assessed incubating the paint samples at optimal conditions for the fungus germination. Finally, the vegetative growth control by the photocatalyst present in the paint was analyzed. This last was achieved treating *A. niger* conidia on a culture medium with or without photocatalytic TiO_2 or Rutile under different types of radiation and observing the surface coverage by the vegetative fungus mycelium.

2. Materials and methods

2.1. Tested paints

Photocatalytic paint composed of water (30%), modified carbon doped TiO_2 Kronos vlp 7000 (18%), $CaCO_3$ (18%), styrene-acrylic Basf Acronal RS 723 resin (33%) and dispersing agent (1%) was employed (**Photocatalytic paint**) [21]. Also, a normal paint was prepared with the same composition but replacing the photocatalytic TiO_2 by the white pigment rutile TiO_2 (Kronos 2360) (**Rutile paint**).

An "antifungal" latex paint (**Antifungal paint**) and a normal indoor latex paint (**Latex paint**), both commercially available in Argentina were also analyzed. It is worth noting that the paint composition of the antifungal paint is not declared by the manufacturer.

2.2. Model microorganism

Aspergillus niger (ATCC 16404) conidia were used for the inactivation assays. This microorganism was selected because it represents the typical environmental fungi in contaminated indoor places, it is non-pathogen, and it is frequently employed as a model in photocatalytic decontamination of environmental molds in air [5,9–16].

2.3. Microorganism culture

Aspergillus niger (ATCC 16404) was revived from conservation beads (where the strain was adsorbed) in malt extract broth and incubated for 72 h at 28 °C. Then, it was cultured on Potato Dextrose Agar (PDA, Merck Chemicals) plate for 7 days at 28 °C (Fig. 1-a). Next, 10 mL of sterile saline solution (0.9%) was used to suspend the conidia that were scraped from the surface culture of PDA. The viable conidia were counted using the surface plate count technique with PDA incubated for 48 h at 28 °C. Also, the obtained conidia suspension was observed under an optical microscope (Trinocular BH2 Olympus microscope, Köhler illumination coupled to LB series objectives, halogen lamps and automatic exposure photomicrography system) to exclude the presence of vegetative mycelium in the suspension. The presence of conidia (highlighted with red circles) and the absence of vegetative mycelium can be observed in Fig. 1-b. Finally, the conidia suspension was stored at 4 °C for 6 months.

2.4. Photocatalytic plates and paint deposition

Borosilicate glass plates of 2 cm × 2 cm were used as support of paint coatings. Before paint immobilization, the glass plates were cleaned-up with soap and water, and then immersed in a solution containing 20 g of potassium hydroxide, 250 mL of isopropyl alcohol, and 250 mL of ultrapure water. The plates were kept in contact with the washing solution for 24 h and then for 2 h under sonication.

The "dip-coating" technique was employed to obtain the photocatalytic films over the glass plates. This method basically consists of immersing the glass to be coated in the paint, and then withdrawing it at a controlled speed. The plates were withdrawn from the paint at a speed of 3 cm min^{-1}, and then dried at 25 °C for 24 h. The quantity of the different deposited paints on the glass plates was determined by weight difference of a large number of plates.

Before the conidia inactivation tests, the coatings were exposed to UV radiation for 24 h. With this procedure the paints were cured, oxidizing the organic compounds that are surrounding the TiO_2 particles in the paint [25] and allowing the interaction between TiO_2 and conidia.

2.5. Experimental setup

The photocatalytic and control experiments were carried out in an experimental setup consisting of a radiation emitting system, an irradiation compartment, and a support to hold the coated glass plates with the paint samples during irradiation (Fig. 2). A borosilicate glass separates the emitting system from the irradiation compartment. The radiation emitting system consists of a set of seven tubular fluorescent lamps T5 of 8 W held by a metallic rectangular box above the irradiation compartment in a horizontal parallel arrangement. Two kinds of fluorescent lamps were employed: cool white visible light lamps (360–720 nm) and UV black-light lamps (300–400 nm). Inside the irradiation compartment, local measurements of the incident radiation flux at different positions on a plane were performed using a radiometer (ILT 1700, International Light Technologies) equipped with visible and UV light sensors for each type of lamp, respectively. More details of the employed experimental setup can be found in [26].

The photocatalytic plates coated with the paint samples were held horizontally in the central zone of the irradiation compartment, where the radiation flux was almost uniform. A saturated solution was included in the irradiation compartment to secure an atmosphere with a constant relative humidity (RH), necessary to obtain sustainable TiO_2 photocatalytic activity. Throughout the experiments, temperature inside the irradiation compartment was kept constant at 30 °C. Furthermore, the relative humidity used in the irradiation assays were 30% and 50%, using magnesium chloride and magnesium nitrate, respectively. A thermohygrometer was employed to measure these variables.

2.6. Microorganism inactivation tests on paints

The inactivation of *A. niger* conidia was evaluated over the previously described paints: Photocatalytic, Rutile, Latex, and Antifungal. The methodology described below is summarized in Fig. 3.

2.6.1. Deposition of Aspergillus niger conidia (Fig. 3-I)

Before starting every inactivation assay, 10 μL aliquots of the conidia suspension of approximately 10^7 CFU mL^{-1} were spread over the coated plates utilizing a micropipette and covering an area of 1.5 cm × 1.5 cm. After that, the plates were maintained at 25 °C in sterile conditions to dry the samples. Irradiation assays were performed with dry conidia to represent the environmental conditions in which

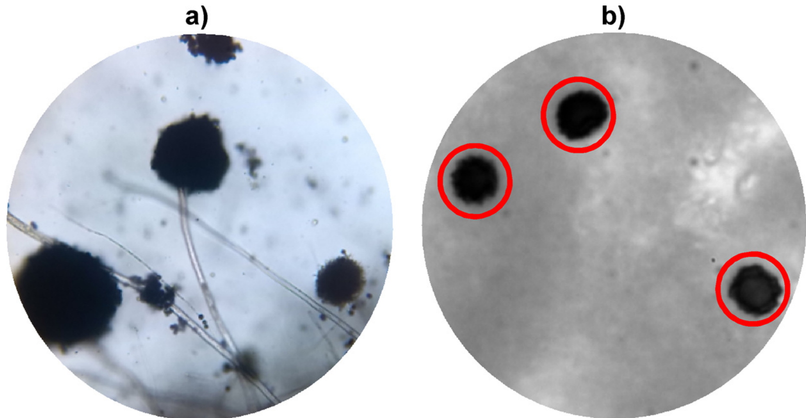


Fig. 1. *Aspergillus niger*. a) Culture micrograph (400 ×). b) Conidia suspension micrograph (1000 ×).

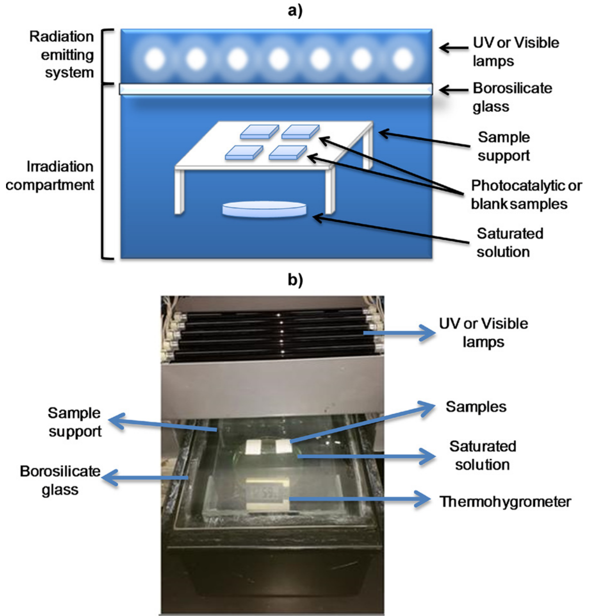


Fig. 2. Experimental setup. a) Schematic diagram of frontal view. b) Picture of superior view.

they are disseminated by air.

2.6.2. Visible or UV irradiation (Fig. 3-II)

The dry plates described above were placed in the irradiation compartment (Fig. 2-a) and exposed to visible or UV radiation during 7 or 3 days, respectively (30 °C, 30% or 50% RH, 100% or 12.5% Irradiation flux, Table 1). After the programmed irradiation time for each sample, the plates were removed and the remaining viable conidia were counted (section 2.6.4). Also, some samples were analyzed with optical microscopy (OM, Olympus BH2) and scanning electronic microscopy (SEM, JEOL JSM-35C, samples coated with gold employing a metal/carbon combined deposition system). Conidia diameters were sized with SemAfore (5.2, JEOL USA, Inc.) passive image digitizer. The program allows to optimize images and to make precise object measurements.

2.6.3. Dark control (Fig. 3-III)

The same procedure described in section 2.6.1 was performed for the samples kept for the same period of time without radiation (in the dark at 30 °C and 30% RH, Table 1). After the programmed irradiation time for each sample, the plates were removed and the remaining viable conidia were counted (section 2.6.4). Each sample was analyzed with optical microscopy (OM).

2.6.4. Counting of viable conidia (Fig. 3-IV)

To perform the counting of viable conidia, each plate (irradiated or dark control) was placed in a tube with 10 mL of sterile extraction solution (0.1% peptone in distilled water). Then, the sample was scraped with a sterile spatula to separate the conidia from the surface of the plate. Subsequently, the tube with the plate, the spatula and the extraction solution were stirred for 3 min. When it was necessary, serial dilutions were made. Finally, aliquots of 0.1 mL of the resulting suspension were spread over the surface of PDA plates, incubated at 30 °C for 48 h, and the CFU counted. The tests were repeated twice for each studied experimental condition, and the counting of the viable conidia of each repetition was made in duplicate.

2.6.5. Theoretical analysis of the experimental results (Fig. 3-V)

The decay of viable conidia as a function of the time was fitted with the following exponential equation [27] and applying the lineal least squares method:

$$N = N_0 \exp(-kt) \quad (1)$$

where N (CFU mL^{-1}) is the conidia concentration at time t, N_0 (CFU mL^{-1}) the initial conidia concentration, k (day^{-1}) an apparent kinetic constant, and t (day) the irradiation time.

2.6.6. Experimental design and factorial analysis of variance (Fig. 3-VI)

The experimental design is a very useful tool from a practical point of view because it reduces the number of experiments to be performed in a study for a certain number of independent variables, taking into account the many possible situations. In addition, it provides a set of statistical and optimization tools in the search for the parameters that best fit the type of process to be performed.

To evaluate the influence of different factors affecting *A. niger* conidia inactivation, a D-optimal factorial experimental design (Design Expert ® Version 7.0.0) was used selecting four categorical factors varied in two levels (Table 1): type of radiation (Vis - UV), relative humidity (30%–50%), type of paint (Rutile - Photocatalytic) and radiation level (100% - 12.5%). The factors significance on the response was analyzed through an Analysis of Variance (ANOVA) and a two-factor interaction model (2FI). ANOVA analysis serves to identify the relevant factors that affect *A. niger* inactivation, allowing to make the substantial improvements in the process. The selected response to perform the factor interaction and level of significance analysis was the first order kinetic inactivation constant fitted applying Eq. (1) to the inactivation experimental data.

2.6.7. Assessment of conidia damage after treatment (Fig. 3-VII)

This experiment was carried out in order to evaluate the damage

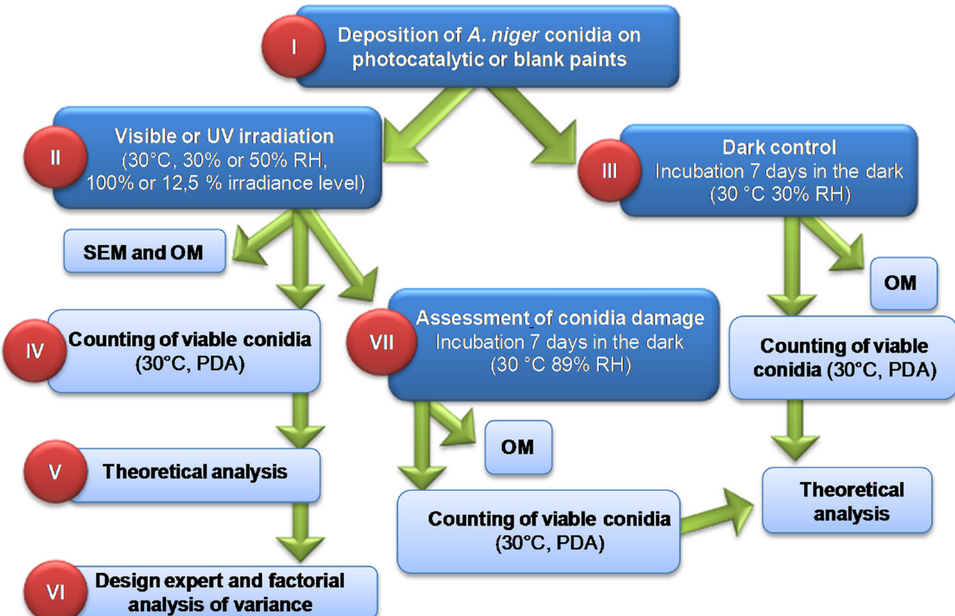


Fig. 3. Methodology description for microorganism inactivation tests on paints.

Table 1
Experimental variables for conidia inactivation on paints.

Paints	Radiation type	RH (%)	Radiation flux (%)	D-Optimal experimental design
Photocatalytic Rutile	Visible UV	50 30	100 12.5	✓
	Dark	30	—	×
Antifungal Latex	Visible	50	100	×
	Dark	30	—	×

caused by visible or UV radiation over the conidia and to analyze the ability to repair it.

For this purpose, the same procedure described in Sections 2.6.1 and 2.6.2 was performed for these assays. The paints used for this study were Photocatalytic and Rutile ones. After the deposition of conidia and the irradiation step applying 100% of visible or UV radiation levels and 30% of RH, the samples were kept for seven days in the dark at 30 °C and 89% RH (saturated solution of potassium sulfate). These conditions are the optimal for the conidia germination, giving the change to repair the damage caused by the irradiation treatment. From the third day of incubation, every two days the sample plates were removed and the remaining viable conidia were counted. The counting of viable conidia was performed as described in Section 2.6.4. Also, each sample was analyzed with optical microscopy.

2.7. Microorganism inactivation tests on PDA, TiO_2 + PDA or Rutile + PDA

With the purpose to verify the vegetative fungi growth control by the active principle used in the photocatalytic paint (anatase carbon doped TiO_2), the methodology shown in Fig. 4 was performed.

2.7.1. Deposition of *A. niger* conidia on PDA, TiO_2 + PDA and Rutile + PDA (Fig. 4-I)

PDA was prepared following manufacturer instructions, but supplemented with 20 g L^{-1} of TiO_2 (Kronos vlp 7000) or pigment rutile TiO_2 (Kronos 2360). Then PDA was autoclaved. Sterile petri dishes with 20 mL of PDA (with or without TiO_2) were prepared and cooled. Each plate was inoculated with 10 µL of *A. niger* conidia suspensions of 10^7 CFU mL^{-1} in three different places over de dish, equidistant each other (Fig. 5).

2.7.2. Visible or UV irradiation (Fig. 4-II)

The petri dishes with the conidia deposition over PDA, TiO_2 + PDA and Rutile + PDA were placed in the irradiation compartment (Fig. 2-a) and exposed to visible or UV radiation during 7 days (30 °C, 30% RH). Petri dish images were taken after 1, 3, 4 and 7 days of irradiation.

2.7.3. Incubation (Fig. 4-III)

After the irradiation assay, the same petri dishes with the conidia deposition were incubated in the dark at 30 °C and 30% RH for 7 days. Petri dish images were taken after 2, 3, 4 and 7 days of incubation.

2.7.4. Dark control (Fig. 4-IV)

The same procedure described in section 2.7.1 was performed for the petri dishes with the conidia deposition over PDA, TiO_2 + PDA and Rutile + PDA kept for the same period of time without radiation (in the dark at 30 °C and 30% RH). Petri dish images were taken after 1, 3, 4 and 7 days of incubation.

2.7.5. Image analysis (Fig. 4-V)

The obtained images were imported into the software ImageTool (UTHSCSA ImageTool Version 3.0), which is a free image processing and analysis program. The percentage of the surface coverage was calculated by applying a specific program which determines the ratio of the black and white pixels. The black pixels correspond to the surface covered with fungi conidia.

3. Results and discussion

3.1. Characterization of paint coatings

An average of 2.29 mg cm^{-2} for Photocatalytic paint deposited on the plates was obtained. On the other hand, when the Antifungal paint was used, the mass per unit area with the same coating technique was 8.50 mg cm^{-2}. Table 2 shows the average specific load for all tested paints.

Also, SEM images were taken from the surfaces of the paints used

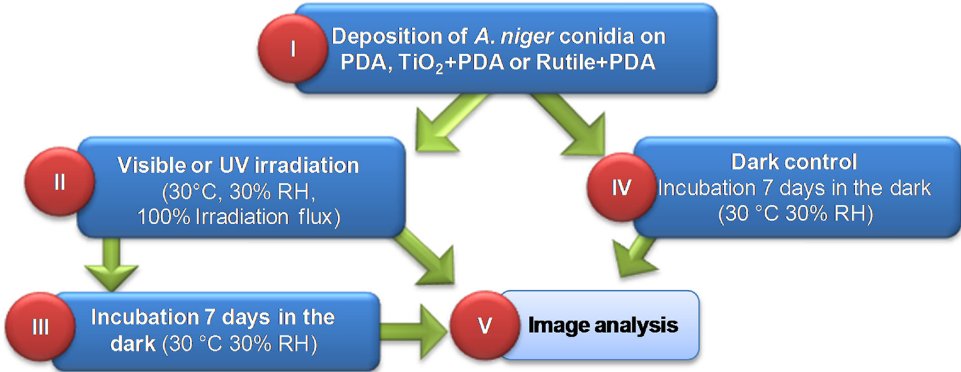


Fig. 4. Methodology description for microorganism inactivation tests on PDA.

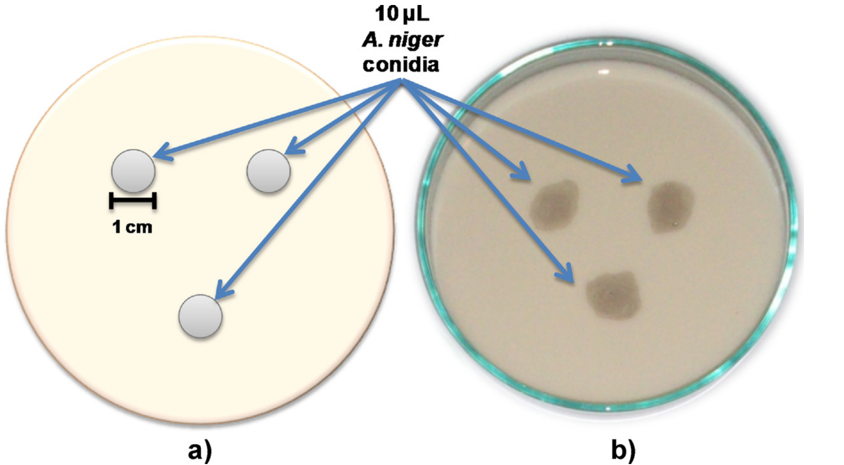


Fig. 5. Deposition of *A. niger* conidia on PDA, TiO_2-PDA or Rutile-PDA. a) Schematic diagram. b) Picture of Rutile + PDA with three conidia deposition.

Table 2
Average specific load on the glass plates of the different tested paints.

Paint	$mg\ cm^{-2}$
Photocatalytic	2.29
Rutile	2.58
Antifungal	8.50
Latex	10.19

before and after irradiation assays. A summary of taken SEM images is presented in Table 3. It can be seen that Photocatalytic paint presents a slight change in the surface after irradiation for 7 days. This change could occur because TiO_2 particles in the paint surface oxidize the styrene-acrylic resin that is surrounding them [25]. However a macroscopic alteration on the paint surface was not detected. On the other hand, Rutile and Antifungal paints do not present appreciable changes before and after irradiation assays.

The surface roughness of the paints coatings was qualitatively analyzed through visual inspection of SEM images and measuring the superficial fissures width (SemAfore 5.2, JEOL USA, Inc., passive image digitizer). Some differences of roughness between samples were observed. However, in any paint coating the size of the superficial fissure (approximately 1 µm) was bigger than the conidia diameter (3–4 µm). Therefore, it could be assumed that the conidia are not trapped in the surface of the paints coatings under this study

3.2. Radiation flux measurement

Using the ILT 1700 radiometer, the incident radiation flux measurements were performed at different positions on the irradiation compartment. Only the most uniformly illuminated surface, placed at the central zone of the irradiation compartment, was employed for the experiments. A value of 6.91 $mW\ cm^{-2}$ for the 100% level of visible irradiation condition between 400–1064 nm was obtained. For the fungus inactivation tests employing ultraviolet light, a value of 1.78 $mW\ cm^{-2}$ for the incident radiation flux between 300–400 nm was measured.

3.3. Microorganism inactivation tests on paints

3.3.1. Inactivation assays (UV, visible and dark)

The inactivation of *A. niger* conidia was evaluated over the previously described paints and over different experimental variables (Table 1), such as radiation type, relative humidity and incident radiation flux, always at 30 °C.

The estimated inactivation kinetic constants applying Eq. (1) (Section 2.6.5) to fit the evolution of viable conidia are presented in Fig. 6. The inactivation constant is a useful parameter to compare the antifungal capacity of different paints working under different conditions. This parameter is independent of irradiation time and initial concentration of fungus conidia, which allows to compare the tests done during different time intervals.

Under visible radiation, after 7 days of treatment, Photocatalytic and Rutile paints presented an average kinetic constant of approximately 0.2 day^{-1} (R^2 = 0.8981-0.9976) under 100% of radiation flux, independently of RH. As expected, when the incident radiation flux was 12.5% a decrease of the constant was observed (about 0.08 day^{-1}, R^2 = 0.7813-0.9626). Because no significant difference between Photocatalytic and Rutile paints was present, it can be concluded that

Table 3
Summary of SEM images of paints coatings (white bar = 1 μm).

Paint	Before irradiation	After 7 days of visible irradiation
Photocatalytic		
Rutile		
Antifungal		

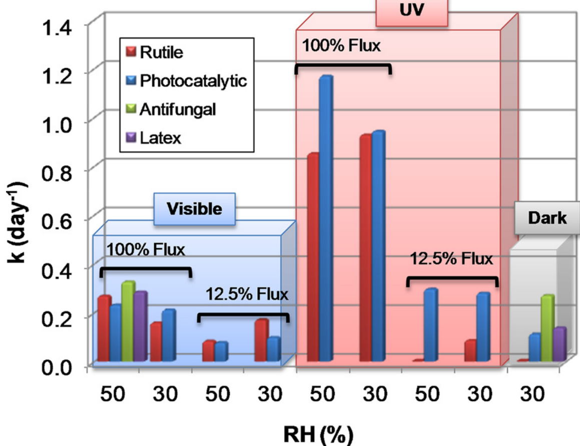


Fig. 6. Kinetic constants of conidia inactivation assays on paints.

the observed inactivation under visible light is mainly photochemical, and the catalytic power of Photocatalytic paint do not show up under these experimental conditions.

It should be noted that Antifungal and Latex paints presented inactivation rates comparable to that of Photocatalytic and Rutile paints under visible light. Therefore, the antifungal performance of commercial first-brand tested paints was similar to the paints designed and prepared in our laboratory. Additionally, when Antifungal paint was kept in the dark at 30 °C for 7 days, as normal working condition of this formulation, the kinetic inactivation constant was 0.270 day^{-1} (R^2 = 0.9854). This result confirms that the main antifungal constituent present on the commercial paint is a chemical fungicide, because a slight difference in the inactivation rate was found with or without light for this paint. In contrast, in the dark control after 7 days without light at 30 °C no inactivation was observed for Rutile, and a very low kinetic constant was obtained for Photocatalytic and Latex paints. Even though, the inactivation by the Latex paint in the dark was higher than the Photocatalytic one since all commercial paints have a fungicide for storage purpose. So, both commercial Antifungal and Latex paint coatings were not used as blank surfaces for the photocatalytic process during the variation of factors of the experimental design.

On the other hand, under UV radiation and after 3 days of treatment, all tested paints presented a kinetic constant 4–5 times higher than with visible light. In particular, the photocatalytic antifungal capacity of Photocatalytic paint (R^2 = 0.8895-0.9759) was evident and higher than the Rutile paint (R^2 = 0.9526-0.9769) with 100% radiation flux and 50% RH and with 12.5% radiation flux for both RH. In this case, the fungus inactivation was due to photocatalytic and photochemical processes. As an example of *A. niger* conidia inactivation on Photocatalytic and Rutile paints under visible and UV light, Fig. 7 shows the evolution of viable conidia applying 100% of radiation level and 50% and 30% of RH, respectively.

3.3.2. SEM and OM

Photocatalytic, Rutile, and Antifungal paints with conidia, before and after irradiation, were observed with Scanning Electron Microscopy (SEM). The results are presented in Table 4. Conidia diameter was sized with SemAfore (5.2, JEOL USA, Inc.) and represents the average of several captured images.

It can be seen that conidia diameter was maintained approximately constant before and after irradiation treatment except under UV radiation with the Photocatalytic paint, where the conidia diameter decreased.

On the other hand, before irradiation the conidia present their typical spherical shape and the spicules form a sharp surface around the

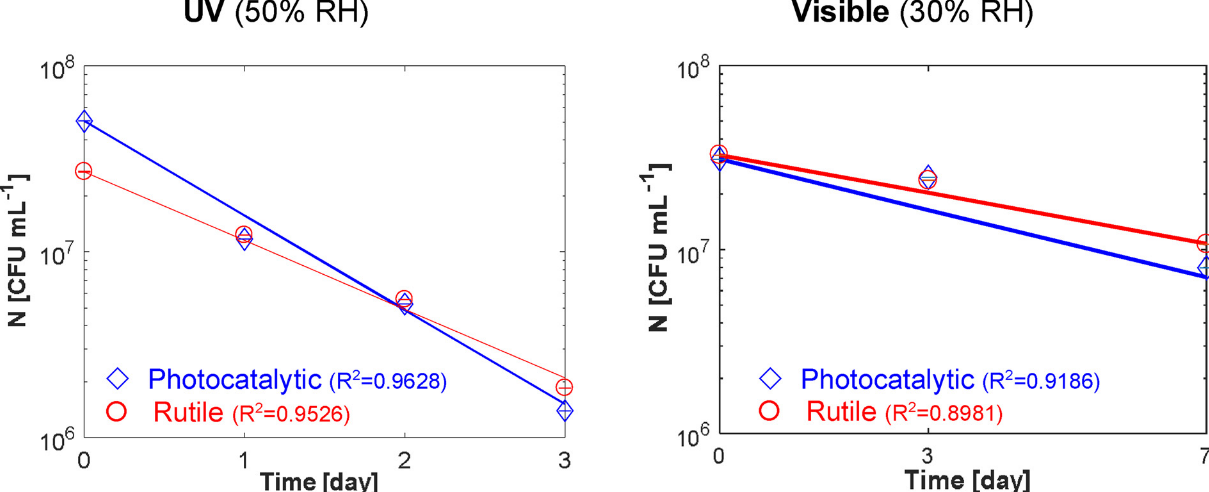


Fig. 7. *A. niger* conidia inactivation on Photocatalytic and Rutile paint under 100% flux of UV and visible light. Experimental values (◇,○) and fitting (——).

conidia surface. After radiation treatment, some conidia have lost the spherical shape, and others present a surface more flattened because the spicules do not present their initial structure. For a better observation of conidia spherical shape loss, red circles were included in Table 4 to highlight the structures referred to.

Also, Photocatalytic and Rutile paints with conidia, before and after irradiation, were observed with Optical Microscopy (OM). Before irradiation the mentioned conidia structure is observed, and the presence of characteristic black conidia pigment is evident. After irradiation, most of this pigment is lost, and this effect is more evident for Photocatalytic paint (Table 5), in which the conidia showed a lighter color than the conidia irradiated over Rutile paint (images not shown).

These morphological changes and depigmentation of *Aspergillus niger* conidia were observed in other works as well [17,28]. In Taylor-Edmonds et al. [28] SEM images revealed that UV irradiated *A. niger* conidia appeared smoother and devoid of any dark structures. This can be related with the high content of melanin in the outer layer of conidia absorbing damaging photons, which gives UV tolerance to fungus. On the other hand, comparing UV-A and photocatalytic treatments, Pokhum et al. [17] have observed in TEM images that in the presence of TiO_2 photocatalyst the *A. niger* melanized layer and cell wall could be destroyed more effective than with UV-A light only. They also found a more deformed conidia shape with photocatalytic treatment. These results can be explained by the oxidative attack of radicals generated during photocatalytic process to the outer layer of conidia cell wall (capsule and spicules). However, the intracellular organelles of *A. niger* conidia were not affected by TiO_2 photocatalysis and UV-A radiation, and only outermost melanin-like layer and cell wall were damaged. This might explain the fact that some damaged *A. niger* conidia can recover their germination.

3.3.3. Experimental design and factorial analysis of variance

The factorial experimental design D-optimal (Design Expert ® Version 7.0.0) was applied considering the categorical factors described in Table 1 for Photocatalytic and Rutile paints. The interaction between them and their significance level for *A. niger* inactivation was analyzed. The following steps were performed:

a) To select a response transformation
b) To choose the significant effects and the factorial model
c) To plot the effects on a normal probability graph
d) To perform an ANOVA for the selected factorial model
e) To check the waste and evaluate the model fit and transformation choice
f) To build the effects graphs to evaluate and interpret the model

The estimated inactivation constant k (Eq. 1) shown in Fig. 6 for each test within the experimental design was selected as the response to carry out the statistical analysis. This parameter allows to make a comparison between experiments of different duration because it is time-independent.

An ANOVA was carried out to statistically verify the significance of the effects (Table 6) applying a two factor interaction model (2FI) and performing any transformation of the response. It is a precise and formal statistical method, which allows knowing, with a certain level of confidence, the effects that significantly influence the response. It is considered a significant effect when the p-value of the factor or interaction is less than 0.05. The p-value is the probability that the null hypothesis is true, that is, a term of the 2FI model does not have influence on the observed results. So, the significant factors obtained in this analysis were: radiation flux, type of radiation and paint, as well as the interactions between the type of radiation/radiation flux and paint/type of radiation. Conversely, the RH change did not show a significant effect on the conidia inactivation.

3.3.4. Assessment of conidia damage after treatment

In order to assess the damage of visible or UV radiation over the conidia and to evaluate the ability of repairing it, Photocatalytic and Rutile paints with conidia were exposed to visible or UV irradiation for 7 and 3 days, respectively (30 °C and 30% RH), and then the samples were kept for 7 days without radiation, in the dark at 30 °C and 89% RH. After 3, 5 and 7 days of incubation, the remaining viable conidia were counted and also each sample was analyzed with optical microscopy to observe possible germination.

Conidia OM images on Photocatalytic and Rutile paints before and after incubation are shown in Table 7. No germination was observed for both paints. During incubation after the visible light treatment (images not shown) conidia did not present any noticeable change. However, for the UV irradiation and over Photocatalytic paint after the incubation time, conidia presented less pigmentation than immediately subsequent to irradiation step (Table 7).

Also, the counted remaining viable conidia and the kinetics analysis (k_r) results are presented in Fig. 8. Here, k_r is a first order kinetic constant for the conidia inactivation during incubation after the irradiation treatment. Eq. (1) was also used to fit the obtained experimental results, but we named the kinetic constant as k_r because the inactivation mechanism that takes place in this assay is unknown (it is not photocatalytic), and cannot be directly compared with the value of the constant previously obtained k (Section 3.1.1).

From these results, we could observe that not only conidia did not germinate but also the inactivation continued even when the irradiation

Table 4
SEM images of Photocatalytic, Rutile, and Antifungal paints with conidia, before and after irradiation.

	Before irradiation	
Conidia diameter	3.736 ± 0.176 µm	
Paint	**3 days under UV**	**7 days under visible**
Photocatalytic		
Conidia diameter	3.420 ± 0.203 µm	3.701 ± 0.279 µm
Rutile		
Conidia diameter	3.840 ± 0.311 µm	3.744 ± 0.252 µm
Antifungal		
Conidia diameter		3.719 ± 0.262 µm

Table 5
OM images of Photocatalytic paint with conidia, before and after irradiation.

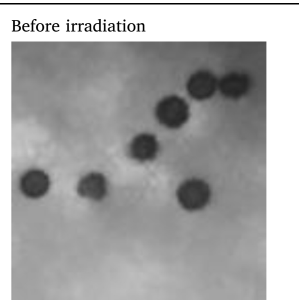
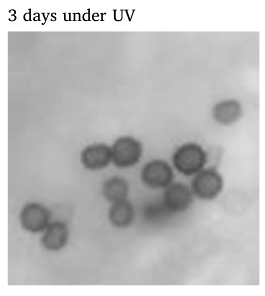
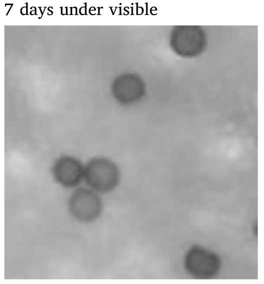
Before irradiation	3 days under UV	7 days under visible
		

Table 6
p-values obtained from ANOVA for two factor interaction model (2FI).

	Factor/Interaction	p-value
	RH	0.5292
✓	Radiation flux	< 0.0001
✓	Radiation type	0.0001
✓	Paint	0.0407
	RH / Radiation flux	0.2529
	RH / Radiation type	0.7539
	RH / Paint	0.4454
✓	Radiation flux / Radiation type	0.0002
	Radiation flux / Paint	0.6061
✓	Radiation type/ Paint	0.0200

assay had already finished. In other words, conidia that had been irradiated over Photocatalytic paint (under visible or UV radiation) presented a residual inactivation kinetic constant k_r much higher than conidia that had been irradiated over Rutile paint (under visible or UV radiation). Also, a dark control was performed. Conidia over on both paints without irradiation exposition were incubated for 7 days in the dark (30 °C and 89% RH). No conidia germination or inactivation was obtained.

It is important to emphasize that these results verify the growth control power of the Photocatalytic paint, no matter what type of light is employed. The Photocatalytic paint not only inactivates the fungi conidia during irradiation but also the damage is such that the inactivation effect continues even after 7 days having completed the irradiation step. Since no such effect was detected in the Rutile paint, it can be assumed that the progressive inactivation in the photocatalytic paint is the result of an irreversible cellular damage produced by the radicals generated previously during the irradiation. Also, this would indicate that the mechanism to repair the damage caused to the *A. niger* conidia structure was insufficient when the microorganism was irradiated on Photocatalytic paint.

There is no mechanism proposed in the literature to explain this "residual inactivation effect". However, the *A. niger* inability to germinate on a photocatalytic surface after irradiation was observed previously in Pigeot Remy et al. [16]. They found that *A. niger* conidia are not able to germinate in photocatalytic AC filters after exposure to UV radiation. In contrast, the initial deleterious effects of UV radiation in absence of TiO_2 coating seem to be reversible and conidia could germinate in the AC filters. This behaviour can be explained with the results of the Pokhum et al. [17], where in the presence of TiO_2 photocatalyst the *A. niger* conidia showed a thinner cell wall and a more deformed morphology compared to normal and UVA-treated *A. niger* conidia. Despite this, damaged *A. niger* conidia could germinate on agar plate under dark conditions.

We can also conclude that none of the paint components can be used by conidia as source of nutrients for their growth under these experimental conditions.

3.4. Microorganism inactivation tests on TiO_2 + PDA and Rutile + PDA

To confirm the antifungal power of the active principle in the photocatalytic paint (TiO_2), *A. niger* growth was evaluated over PDA, TiO_2 + PDA and Rutile + PDA under different irradiation conditions (Fig. 4), during 7 days at 30 °C and 30% RH. Petri dish images were taken during irradiation and incubation steps (Table 8). The images were imported into the free software ImageTool (UTHSCSA ImageTool Version 3.0). The covered surface percentage was calculated determining the black and white pixels percentage. Black pixels correspond to the fungal conidia covered surface. The results are presented in Fig. 9.

Under visible light, after 1 day of irradiation minor changes were observed. However, after 7 days of irradiation, TiO_2 + PDA presented a much lower fungi growth (33% of black pixels) than Rutile + PDA (91% of black pixels) or PDA (98% of black pixels). Here, the growth control effect of the TiO_2 included within the Photocatalytic paint was evident.

Under UV light, after 7 days of irradiation, almost no growth and no relevant differences between the three culture media were observed. So, this could be assumed as the result of the photochemical effect produced by UV. On the other hand, after 2 days of incubation, the fungi growth was much slower for TiO_2 + PDA (29% of black pixels) than for Rutile + PDA (96% of black pixels) or PDA (81% of black pixels).This demonstrates again the residual growth control effect of TiO_2.

It is important to evaluate the results obtained under dark control.

Table 7
OM images of Photocatalytic and Rutile paints with conidia treated with UV radiation, before and after incubation assay.

Radiation	Paint	
3 days UV	Photocatalytic	Rutile
Before incubation		
After incubation		

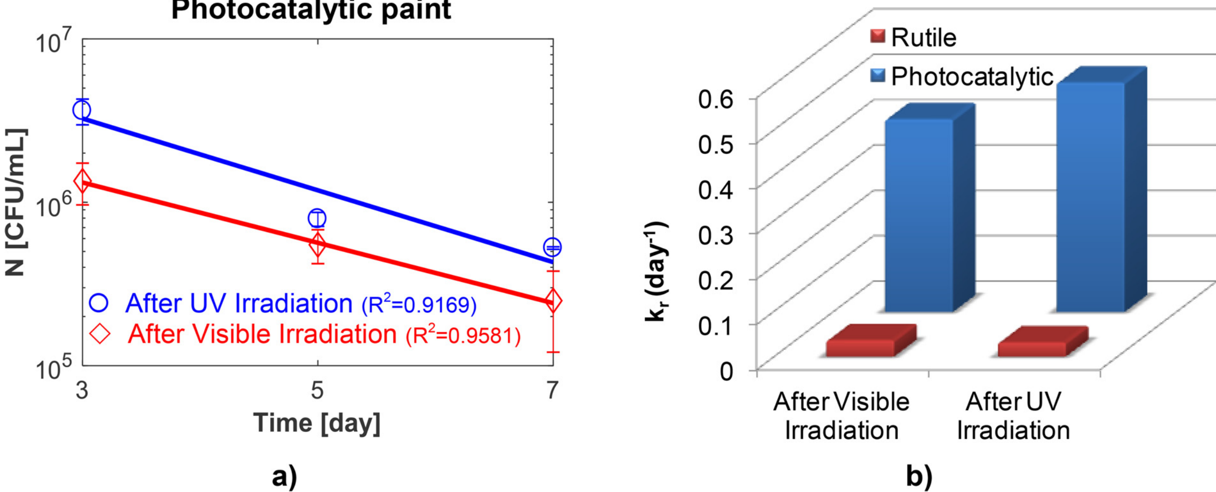


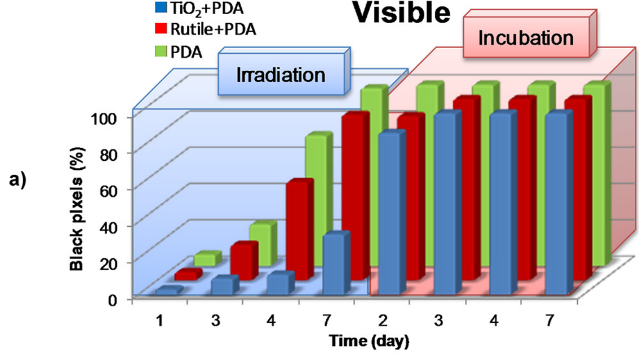
Fig. 8. Assessment of conidia damage after irradiation treatment during incubation assays (30 °C, 89% RH): (a) Remaining viable conidia (experimental values (◇,○) and fitting (——)), (b) Kinetics analysis.

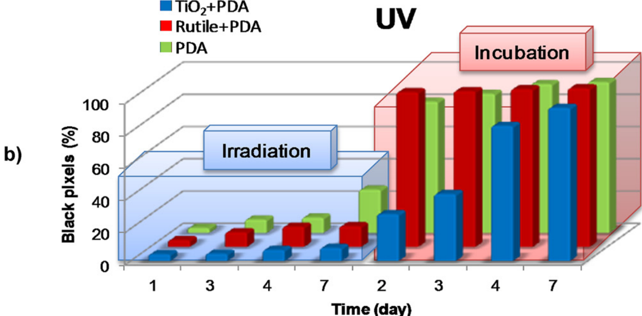
Table 8
Petri dish images taken during microorganism destruction tests on TiO2 + PDA, Rutile + PDA and PDA.

Visible		**Time (day)**	**TiO_2 + PDA**	**Rutile + PDA**	**PDA**
Irradiation		7			
Incubation		2			
UV		**Time (day)**	**TiO_2 + PDA**	**Rutile + PDA**	**PDA**
Irradiation	7				
Incubation	2				
Dark control	**Time (day)**		**TiO_2 + PDA**	**Rutile + PDA**	**PDA**
Incubation	1				
	7				

We could notice that with TiO_2 + PDA the fungi growth was delayed in comparison with Rutile + PDA or PDA. Nevertheless, from the images taken during day 1 to 4 we observed that *A. niger* formed vegetative white micelium only (picture not shown) in the whole plate, and the reproductive structures (conidia) were absent. This is the reason why the image analysis software was not able to quantify the growth. Just at day 7 in the dark the conidia were present. It is evident that TiO_2 presence generated some reproduction delay. However, this result did not oppose or invalidate the results obtained above with Visible or UV light, where the growth control effect was demonstrated.

On the other hand, it is important to point out that the residual inactivation after irradiation treatment obtained previously (sec. 3.3.4) was not so evident here, particularly working with visible light. This could be explained by the fact that the PDA medium supplied the nutrients for conidia recovery and DNA reparation.





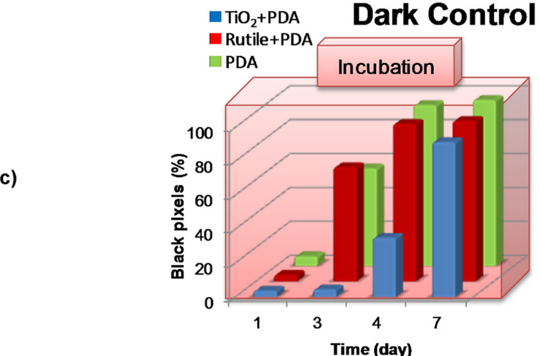


Fig. 9. Covered surface percentage calculated under irradiation and subsequent incubation: a) Visible light, b) UV light and c) Dark control.

4. Conclusions

The inactivation of *A. niger* conidia was evaluated over a Photocatalytic paint (anatase carbon doped TiO_2), a homemade normal Rutile TiO_2 paint, a commercial Antifungal paint and a commercial Latex paint, applying different experimental variables (radiation type, relative humidity and incident radiation flux).

Under visible radiation, after 7 days of treatment all tested paints, Photocatalytic, Rutile, Latex and Antifungal, presented a similar kinetic constant. It could be assumed that the observed inactivation was mainly a photochemical inactivation. Moreover, it could be concluded that the main antifungal constituent present in the commercial paint was a chemical fungicide, since a slight difference in the inactivation rate was found with or without light for this paint.

In addition, under UV radiation all tested paints presented a higher kinetic constant than with visible light, and in particular the kinetic constant of Photocatalytic paint was higher than the constant of the Rutile paint. In this case, photocatalytic and photochemical processes were taking place. These results also were confirmed with Scanning Electron Microscopy and Optical Microscopy.

Besides, to evaluate the influence of different factors affecting *A. niger* conidia inactivation, a D-optimal factorial experimental design (Design Expert ® Version 7.0.0) was applied. The significance and factors interaction on the selected response (the inactivation kinetic constant) through an ANOVA and using a two-factor interaction model (2FI) was analyzed. The significant factors obtained were: radiation flux, type of radiation and paint, as well as the interactions between the type of radiation/radiation flux and paint/type of radiation.

From the assessment of conidia damage after irradiation treatment, it could be observed that conidia inactivation (irradiated over Photocatalytic paint under visible or UV radiation) continued even after the completion of the irradiation assay. These conidia presented damage in their structure that could not be repaired by *A. niger* during the incubation time. These results validated the growth control power of the Photocatalytic paint under both type of radiation.

Finally, the antifungal power of the tested paints was confirmed by verifying the capacity of the active principle used in the photocatalytic paint (carbon doped TiO_2) to control the vegetative fungi growth. For this purpose, *A. niger* over PDA (Potato Dextrose Agar), TiO_2 + PDA and Rutile + PDA was exposed under different irradiation conditions. A delay of *A. niger* mycelium growth on TiO_2 + PDA was found under UV and visible radiation.

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