Acta Polytechnica CTU Proceedings **40**:93–97, 2023
https://doi.org/10.14311/APP.2023.40.0093

Published by the Czech Technical University in Prague

APPLICATION OF COLD ATMOSPHERIC PLASMA FOR MOLD INACTIVATION

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Abstract. Mold growth in indoor interiors is an increasing problem that has adverse effects on both occupants and building materials. The aim of this study was to present the new environmentally friendly method (cold atmospheric plasma treatment) for decontamination of indoor environments contaminated by mold spores, with the potential to become an effective replacement for conventional methods, such as ultraviolet-C (UV-C) germicidal irradiation. The spores of Aspergillus species on malt extract agar plates were exposed to plasma produced by diffuse coplanar surface barrier discharge. The surface coverage of mold was evaluated using image analysis. Cold plasma treatment inactivated spores on the surface of the culture medium in 0.5 min, whereas a weak growth delay was observed after 30 minute exposure to UV-C irradiation. This study demonstrated that cold atmospheric plasma is a more effective method for reducing mold spores on agar compared with germicidal UV-C irradiation.

Keywords: Cold atmospheric plasma, mold, spore, inactivation, mold growth curve.

1. Introduction

People spend most of their time in buildings. The presence of mold contamination in indoor environments has adverse health effects as a result of inhaling mold spores and microbial volatile organic compounds produced from mold metabolism. The most common health risks related to mold exposure are eye, skin, and respiratory tract irritation and also activation of immune responses through the inhalation of airborne mold spores. Their concentrated presence in the indoor environment can cause degradation of the building material and changes in the visual appearance of the surfaces [1]. The major factors affecting indoor mold growth are temperature (between 15 and 30 °C), moisture, and nutrients. Cladosporium, Aspergillus, Penicillium, and Alternaria are identified as the predominant mold species in indoor environments [2].

One of the effective ways to manage mold in a building is to eliminate or limit mold spores and their growth. For microbiological decontamination/inactivation, many methods (mechanical, chemical and physical) and technologies (e.g., UVGI technology, HEPA filtration technology) are used. They are mostly based on the application of chemical substances that are often toxic [3]. Furthermore, some spores are highly resistant to physical and chemical agents. In recent years, cold (non-thermal) plasma has emerged as effective sporicide against a wide spectrum of bacterial and fungal spores [4, 5].

Cold atmospheric plasma (CAP) is widely used in various applications such as surface modification, inactivation of pathogens, degradation of toxins, water purification, etc. [6, 7]. Cold atmospheric plasma is known for its decontamination and disinfection effects against bacteria, viruses and fungi [4]. Several studies have reported that CAP can effectively inactivate microorganisms in the food industry, medicine (e.g., prevention of food spoilage, foodborne diseases, sterilization of medical devices) [8]. CAP also seems to represent a possible alternative method to standard techniques for the inactivation of mold spores, bacteria, viruses, mycotoxins and fungi in the indoor environment or the mold growth on the surfaces of building materials. CAP is an ionized gas that contains a wide range of energetic species, such as reactive oxygen and nitrogen species (RONS), free electrons, neutral molecules, and ultraviolet radiation. Cold atmospheric plasma can be generated from air at atmospheric pressure. Atmospheric plasma equipment is significantly cheaper compared to low pressure plasma system because it does not require expensive vacuum chambers and pumps. Plasma technology does not use toxic chemicals. These make plasma treatment very fast, economically advantageous and environmentally friendly [8].

The discharge methods for generating CAP mainly include, plasma jets, dielectric barrier discharge, floating-electrode dielectric barrier discharge and corona discharge [8]. The effectiveness of CAP in inactivating spores/microorganisms is affected by various factors such as plasma reactor type, device setup, gas composition, operating conditions (e.g., input power) and treatment duration.

Ultraviolet-C (UV-C) light is a short wavelength (200–280 nm) radiation and is primarily used for the

Figure 1. Photo of plasma treatment of Aspergillus brasiliensis on malt extract agar plates using diffuse coplanar surface barrier discharge (DCSBD) in ambient air.

disinfection of air and surfaces from microbial contaminants.

In this study, the efficacy of mold spore inactivation by UV-C irradiation and cold atmospheric plasma is demonstrated.

2. Materials and methods

2.1. Mold and growth conditions

The mold strain used in this study was Aspergillus brasiliensis CCM8222 (ATCC 16404). A volume of 0.1 ml of spore suspension (26 microrganisms/0.1 ml) was inoculated onto one surface of each test specimen using a pipette. The aim of pipetting the suspension on the surface was to inoculate the spore suspension in the center of the Petri dishes (diameter 90 mm). In an effort to minimize contamination with spores and dust, the inoculation was conducted in a laminar airflow cabinet and the test specimens were manipulated with gloves. After inoculation, the samples were incubated for 14 days under constant conditions at 23 °C and 86.7 ±1.7 % relative humidity.

2.2. Mold inactivation

The inoculated samples were treated in a diffuse coplanar surface barrier discharge (DCSBD) developed by Robust Plasma Systems (RPS400; Roplass, Czech Republic). The DCSBD consists of a large number of microdischarges and generates a plasma layer with approximately 0.3 mm thickness on the surface of the planar electrode. The area of the planar electrode connected to the HV power supply is 80 × 200 mm. The frequency of supply voltage is usually in the range of 14–18 kHz and the corresponding discharge input power ranges from 80 W up to 400 W. The specific property of the DCSBD plasma is the high plasma power density of 100 W · cm^{-3} and the homogeneity of the DCSBD plasma increasing with the discharge power density. The DCSBD plasma source was applied to the surface of samples with spores for an exposure time ranging from 0.5–10 minutes with a power of 300 W, and to the surface of samples with young mycelium (treated 72 hours after inoculation) for an exposure time of 10 min with a power of 300 W. All samples were treated homogeneously. The plasma treatment of the sample is shown in Figure 1.

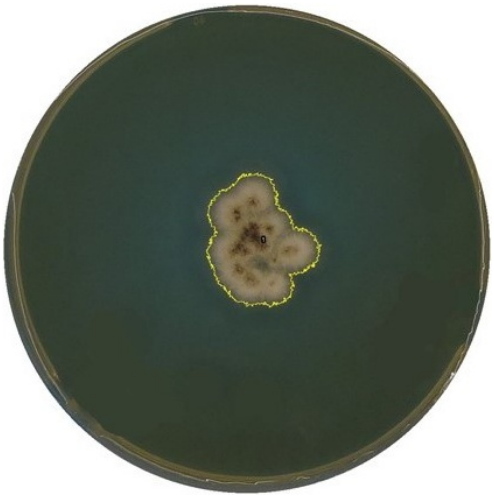

Figure 2. Image analysis of Aspergillus brasiliensis on malt extract agar plates using FIJI software.

Ultraviolet (UV-C) irradiation was performed using a germicidal UV-C lamp (LB 301.2, 2 × 30 W, tubes length 920 mm, wavelength 253.7 nm). The intensity was approximately 450 µW/cm^2 across the exposure area. All samples in the UV-C irradiation group were exposed for 30 min and the distance between the lamp and the samples was 30 cm.

2.3. Mold growth assessment

The mold coverage on each of the plates was determined by image analysis using the open-source FIJI software. A digital photograph of the samples was taken using a Canon G1X camera. An ISO speed (camera sensor's sensitivity to light), a shutter speed, and an aperture were set to 800, 1/100 s, and $f/5.6$, respectively. All pictures of the Petri dishes were taken under the same lighting conditions to avoid reflections from the Petri dishes surfaces. A per-image thresholding method was used to detect the mold growth from its background (growth media) and resulted in a more accurate measurement of mold area coverage (Figure 2). ImageJ is a useful tool for evaluating mold growth on malt extract agar plates. Image analysis provides an objective evaluation and improves the comparability of results. The accuracy of the image analysis was increased by using the same lighting conditions.

Mold growth on the inoculated surface of each test sample was evaluated once a day for 14 days. The percentage of mold coverage C was calculated according to Equation (1):

$$C = \frac{A_M}{A} \cdot 100\%, \tag{1}$$

where

A_M is the area of the plate surface that was covered by mold,

A is the total area of the Petri dish.

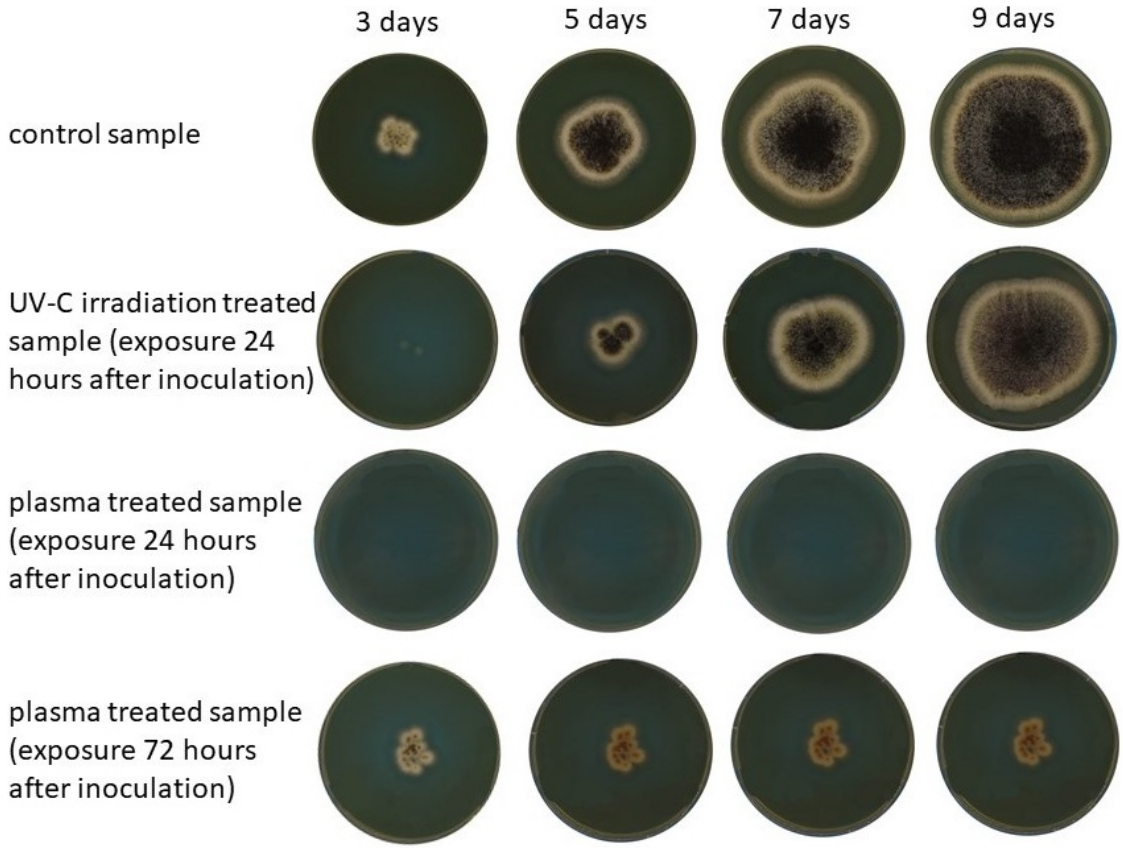

FIGURE 3. Mold growth of Aspergillus brasiliensis on malt extract agar plates. Representative pictures were taken 3, 5, 7 and 9 days after inoculation: (a) control sample, (b) UV-C treated sample (exposure time 30 minutes, treated 24 hours after inoculation), (c) plasma treated sample (300 W, exposure time 0.5, 1, 5 or 10 minutes, treated 24 hours after inoculation), (d) plasma treated sample (300 W, exposure time 10 minutes, treated 72 hours after inoculation).

3. Results and discussion

Using Aspergillus brasiliensis spores as a target organism, a comparison of low temperature-based decontamination techniques is reported. The inactivation of A. brasiliensis was performed using CAP and UV-C irradiation and was determined by image analysis of the mold growth area in Petri dishes. There were six treatment groups and one control group (Figure 3). In the case of control group, A. brasiliensis grew rapidly after inoculation and the mold completely covered the Petri dishes after 12 days. The mold growth was noticeable 2 days after inoculation, the molds grew from the center to the outer edge and formed a circular colony with irregular edges (mold spores were inoculated at the center of the nutrient). The control sample (Figure 3) shows the mold growth phases. Initial spore inoculation is followed by lag phase (spore adhesion), spore germination, hyphae growth and reproduction. During the lag phase, the spores remain inactive until they have absorbed sufficient moisture and nutrients from the substrate. Hyphae are filaments that appear immediately after germination, and as they thicken, they form a mass called mycelium. Colonies of A. brasiliensis were initially yellowish and then dark brown to black. In Figure 3, morphological differences were observed between the control and CAP treated samples, such as color changes and colony growth arrest. Cold plasma treatment of the samples (72 hours after inoculation) for 10 min with a power of 300 W completely inhibited spore germination and hyphal growth of A. brasiliensis. As demonstrated in Figure 3, 30-minute UV-C treatment, slightly prevented the colonization of A. brasiliensis on samples. In inactivation spore experiments (24 hours after inoculation), plasma treatments were performed for 0.5, 1, 5 and 10 minutes at 300 W. Plasma treated samples did not show mold growth during 14 days.

The biological effects of plasma treatment have been demonstrated in several studies [4, 8, 9]. Several important mechanisms occur in the process of plasma inactivation, such as chemical reactions with atomic oxygen, and UV-induced damage. The microbial inactivation of plasma is mainly attributed to RONS. RONS include O_3 and H_2O_2, which are powerful oxidants and have similar inactivation mechanisms. These reactive species inactivate molds by progressive oxidative damage to their cell walls and membranes. UV radiation, which is also produced during the formation of CAP, is not considered to be a dominant factor in the inactivation process.

The results of mold growth image analysis during 14 days of incubation are depicted in Figure 4. All data are presented as the mean ± standard deviation.

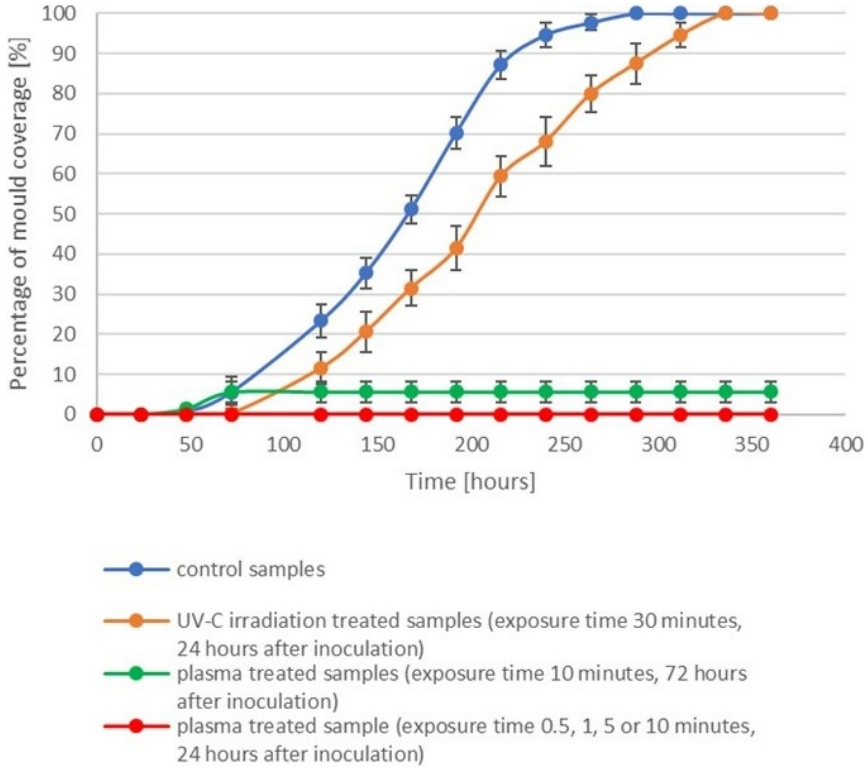

Figure 4. Mold growth curves of Aspergillus brasiliensis on malt extract agar plates.

The mold in the control samples grew according to the typical s-shaped curve (logistic growth). The lag phase lasted two days. Thus, the measurements started on the second day and continued until the 14th day. The exponential growth went up to the 7th day (inflection point) and it was possible to observe a color change, to a black tone in the center of colonies.

Compared to the control group, mold growth was negligibly suppressed by the UV-C germicidal lamp. As shown in Figure 4, the coverage area of Aspergillus brasiliensis treated with UV-C irradiation was slightly smaller than that of the control group at the same time. Spore color can play an important role in inactivation of mold spores by UV-C irradiation. The Aspergillus brasiliensis spores are more resistant, in that the pigment of the A. brasiliensis spores absorbs more in the UV-C region and thus protects the spores from UV irradiation [10]. The growth curve results also clearly indicate that the 30-second CAP treatment (24 hours after inoculation) was sufficient to completely inactivate the A. brasiliensis spores and prevent mold growth in all samples. The effect of CAP treatment on permanent inactivation of A. brasiliensis spores was also evaluated for longer exposure times (1–10 min). Based on the above results, plasma treatment is considerably more effective than a conventional UV-C irradiation. CAP treatment achieved a high level of inactivation level within a short time.

4. Conclusion

In this study, it can be concluded that the cold atmospheric pressure plasma has potential sporicidal activity against molds and could be a promising tool for the inactivation of spores in indoor environments or on the surface of building materials. The cold atmospheric plasma treatment was compared with a conventional germicidal UV-C lamp, which is commonly used to reduce mold spores in indoor environments. The results of image analysis of mold growth on malt extract agar plates treated with germicidal UV-C lamp showed a non-significant delay in mold growth dynamics. The appearance of the first mold colonies exposed to UV-C irradiation was delayed by only 24 hours compared to the control samples. Furthermore, 0.5 min of CAP treatment (24 h after inoculation) was shown to lead to inactivation of A. brasiliensis spores. The inactivation of young mycelium (hyphae) was also tested by cold atmospheric pressure plasma treatment. Images of samples with young mycelium exposed to plasma produced by diffuse coplanar surface barrier discharge for 10 minutes at 300 W (72 hours after inoculation) show an obvious change in mold color and an arrest of growth. Compared to control samples, it was observed that cold atmospheric pressure plasma treatment caused inactivation of spores and young mycelium growth. The mode of CAP action on microorganisms is still the subject of extensive studies, but it is believed that biologically reactive substances generated by plasma (RONS, UV radiation, and charged particles) act cooperatively to inactivate microorganisms. However, more studies are needed to understand in detail the mechanism of spore inactivation and young mycelium growth by cold atmospheric plasma.

Acknowledgements

This work was supported by the Czech Science Foundation (No. GA22-06621S).

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