Parametric study of afterglows issued from Dielectric Barrier Discharges in nitrogen and air and applied on *Aureobasidium* fungi

E Lecoq\(^1\), C Leclaire\(^2\), F Clément\(^1\), G Orial\(^2\), E Panousis\(^1\) and A Ricard\(^3\)

\(^1\)LEGP - IPREM - Université de Pau, 64000 Pau, France
\(^2\)LRMH - 29 rue de Paris, 77420 Champs-sur-Marne, France
\(^3\)LAPLACE - Université Paul Sabatier, 31000 Toulouse, France

**Abstract.** This article is devoted to the study of afterglows issued from two Dielectric Barrier Discharge reactors driven by two distinct High Voltage generators. The obtained afterglows are applied on *Aureobasidium pullulans* fungi in view of a curative treatment. In a first part, the electrical characteristics of the discharges obtained with both systems are studied and in a second part are presented the results of the fungi treatment efficiency.

1. Introduction

Last years, biological decontamination by plasma discharges or by plasma afterglows has shown very promising results [1–3]. In most of the cases, treatments are applied on bacteria such as *Escherichia coli* or *Bacillus subtilis*, but any study about the inhibition of fungi via plasma treatments was found. Thus, our study consists in applying afterglows issued from Dielectric Barrier Discharges (DBDs) on the *Aureobasidium pullulans* fungi, which is one of the so-called “blue-stain fungi”, responsible for the bluish discoloration of wood. These discolorations do not alter wood mechanical properties but can cause heavy economical losses in sawmills or also to deteriorate some historic monument materials and in that way to destroy parts of our cultural heritage.

The main objective is, for a first step, to set up the experimental device and the characterisation means and to find the best parameters for the wanted curative treatments. Consequently, the efficiency of afterglow decontamination is initially assessed directly on fungal cultures, set on a glass slide, in order to isolate it from the wood substrate and to try to understand the mechanisms involved.

2. Experimental set-up

Figure 1 shows a schematic representation of the electrical set-up. The gases used for the study are mixtures of nitrogen and oxygen, the oxygen percentage being comprised between 0 and 20%. Gas flows are controlled by gas flowmeters at the inlet of the reactor, and the total flow value was kept constant at 80 slm for the whole study. The applied voltage is measured by a 1/1000 Tektronik P6015A voltage probe while the total current flowing in the circuit is measured by a Rogowski-type current probe (Tektronix CT-2). The electrical signals are visualized by means of a Tektronix 3054B oscilloscope (500 MHz, 5 Gs/s). The oscillograms obtained permit the calculation of the average plasma power over a period of the applied HV signal.

In the study, two distinct systems of DBD reactors and HV generators were used and are described in the following parts. In each case, an adaptor is added to the reactor in order to collect the gas exiting from the reactor and to guide it in a quartz tube of 1 cm in diameter. This way, the afterglow is applied.
on the sample which is placed at 1 cm from the exit of the tube. The sample moves thanks to a custom-
made conveyor belt so that the treatment is dynamic and the entire surface can be treated. The duration of the treatment is then defined by the velocity of this conveyor belt.

Figure 1. Scheme of the experimental set-up.

2.1 Industrial reactor driven by a quasi-sinusoidal HV generator

The first DBD reactor is an industrial one and is schematically represented on figure 2. It presents a cylindrical coaxial geometry. The HV signal is applied on the inner electrode which is coated with a dielectric, while the outer electrode is grounded. The gas enters through the upper slit, circulates around the inner electrode and exits through the lower one forming a rectangular afterglow which is channelled in the quartz tube. The surface of the discharge is 56.4 cm².

This reactor is electrically supplied by a generator which delivers quasi sinusoidal voltage and current waveforms in a frequency value of about 125 kHz in our conditions. An external trigger has been added to this generator in order to chop the signal and to form voltage ON and voltage OFF phases enhancing the variation of the duty cycle. A more detailed description of this generator and of the chopping system can be found in [4].

Figure 2. Schematic representation of the industrial reactor used for the first system.

Figure 3. Schematic representation of the custom-made reactor used for the second system.

2.2 Custom-made reactor driven by a pulsed HV generator

For the second system, a custom-made reactor presented on figure 3 was used. As for the industrial one, it is a reactor with a cylindrical coaxial geometry. The coating (alumina) is on the inner electrode, where the high voltage is applied, and the outer electrode is grounded. The gas flow is axial and, again, an adaptor enhances the guidance of the afterglow in the quartz tube. Rogowski profiles have been produced on this reactor in order to avoid parasite discharges. The surface of the discharge obtained with this reactor is equal to 4.15 cm².
This custom-made reactor is electrically supplied by a generator which delivers positive and negative impulses. The actual signal presents residual oscillations, but it is nevertheless possible to form ON and OFF phases which allow working in pulsed-type conditions. For a detailed analysis of this pulsed HV generator the reader can refer to [5].

3. Treatment electrical conditions

The treatment electrical conditions were defined for each system in a way to have the highest power density and a temperature of the afterglow inferior to 40° Celsius. Those electrical conditions were kept constant for the whole study and the studied parameters were the percentage of oxygen in the gas mixture and the treatment duration.

With the sinusoidal HV generator, the parameter on which we can play is the total delivered power. It was fixed to 900 Watts. In order to keep the temperature inferior to 40° Celsius, the duty cycle was adjusted to 10%. With the pulsed-type generator, we can vary the frequency and the applied voltage. To be in agreement with our conditions described previously, the frequency value was adjusted to 120 kHz and the maximum applied voltage was around 4 kV.

In order to study the electrical properties of the discharge, the applied voltage and the total circuit current are measured and visualised on an oscilloscope and from Kirchhoff’s equations the conduction current ($I_z$) and the gap voltage ($V_g$) are calculated and the plasma power can be determined. Figures 4 and 5 represent the typical conduction current, gap voltage, and plasma power waveforms obtained for each system.

![Waveforms](image.png)

**Figure 4.** Waveforms of (a) gap voltage and conduction current, (b) plasma power obtained with the first system (on one period of the applied voltage).

**Figure 5.** Waveforms of (a) gap voltage and conduction current, (b) plasma power obtained with the second system (for the positive pulse of the applied voltage, the negative one is similar).

Mean plasma powers are, respectively, around 800 and 40 W for the first and the second system but in view of a comparison between the two systems, the surface of the discharge has to be taken into account. That is why mean plasma power densities were estimated following the formula (1):

$$<P_{dens}>=<P>/<S>,$$

(1)
where \( <P> \) represents the mean plasma power in watts calculated on one period of the applied voltage and \( S \) is the surface of the discharge in cm². Thus, plasma power densities were estimated to be around 15 W/cm² for the first system and 10 W/cm² for the second one in those electrical conditions.

In view of the application, the parameter which will be representative is the mean energy density injected during the treatment. Energy densities were calculated taking into account the duty cycle \( \tau_c \), the mean plasma power density \( <P_{dens}> \), and the treatment duration \( t \) as in formula (2):

\[
<E_{dens}> = \tau_c \times <P_{dens}> \times t.
\] (2)

For the industrial reactor driven by sinusoidal HV generator, the duty cycle is equal to 10%. Conversely, for the custom-made reactor driven by pulsed HV generator we can consider that the duty cycle is equal to 1 because the mean plasma power density is calculated on one period of the applied voltage, applied 100% of the time. Figure 6 represents the value of mean energy densities injected for both systems as a function of treatment duration.

It can be observed that the mean energy density injected for a given treatment duration is much higher with the custom-made reactor driven by pulsed HV generator than with the industrial reactor driven by sinusoidal HV generator.

4. Ozone measurements

As already mentioned, treatments have been conducted with gas mixtures containing between 0 and 20% of oxygen. As a result, when oxygen is introduced in our system (<1%), the afterglow is not luminescent and optical emission spectroscopy can not be used to study the species present in the afterglows. However, ozone measurements can be conducted, using the absorption properties of this molecule. The objective is here to compare the ozone production as a function of the oxygen percentage for both systems, knowing that ozone can be one (but not the only one) of the chemical species responsible of living cell damaging.

4.1 Experimental set-up

The gas exiting from the quartz tube is brought in a measurement unit (\( l = 5 \) cm). A mercury lamp is used and a filter enhances the selection of the 254 nm wavelength, for which the ozone absorption cross-section is the highest. The transmitted intensities are measured at the exit of the measurement unit via a photomultiplier. The ozone number density is calculated using the ozone absorption cross-section at 254 nm and the Beer-Lambert law.

4.2 Results

When the discharge is ignited, we can observe a progressive increase of the ozone density in the measurement cell. After a few minutes, a plateau is reached. Figure 7 represents the ozone densities measured after having reached this steady state as a function of oxygen percentage with both systems.

For the first system, the ozone production first increases with the oxygen proportion, but after 10%, it seems that a plateau is reached. For the second system, the ozone production seems to be constant.
whatever the oxygen proportion is but the obtained densities are lower compared to the maximum values obtained with the first system.

![Figure 7. Maximum ozone densities reached as a function of oxygen percentage in the mixture with both systems.](image)

5. Samples preparation
Samples were prepared from ten-day fungi cultures of *Aureobasidium pullulans* which were mixed with 20 ml of sterile water. Then, 100 µl of this liquid culture were spread on microscope slides and let to dry at ambient temperature before being exposed to the afterglow.

6. Characterisation means
After treatments, two characterisation means were used to observe the effect or the afterglow on the fungi: fluorescence microscopy and synthetic culture medium.

6.1 Fluorescence microscopy
The fluorescence microscopy is a technique that can give information about cells viability. Cells are stained with fluorescent dyes: fluorescein diacetate (FDA) and propidium iodide (PI). When observed with a fluorescent microscope, these dyes will, respectively, fluoresce green and red. FDA is used as a viability probe since enzymatic activity is required to activate its fluorescence and because cell-membrane integrity is necessary for its intracellular retention. On the contrary, PI is used for the detection of non-viable cells: it can be taken up only by cells which are dead or have their membrane damaged. Consequently, when cells are observed with fluorescent microscope, viable and non-viable cells, respectively, fluoresce green and red [6]. It is important to remark that this technique can only be used as a qualitative one.

6.2 Synthetic culture medium
The synthetic medium culture is used to observe how fungi can develop themselves and to grow after having been exposed to the afterglow. After treatments, microscope slides are rubbed with a sterile swab which was applied on malt agar plates. After incubation at 24°C during 4 days, colonies are formed and counted in colonies forming unit (CFU). By this way, it is possible to compare the growth of treated fungi with controls.

7. Results
Four runs of experiment were conducted. Table 1 represents the results obtained for the first run. Treatments were conducted with the industrial reactor driven by quasi-sinusoidal HV generator. Treatment duration was constant (15 minutes) and the energy density was consequently equal to 1350 J/cm². Gas mixtures containing 0, 5 and 20% of oxygen were tested. Results show that after a treatment conducted with a gas mixture containing 5% of oxygen, few viable cells can be observed with fluorescent microscopy and that any colonies are formed on synthetic culture medium, denoting a good efficiency of the treatment.
Table 1. Results of fluorescence microscopy and synthetic medium culture obtained for the first run of treatments.

| Gas mixture N₂/O₂ | 100/0 | 95/5 | 80/20 | Control |
|-------------------|-------|------|-------|---------|
| Fluorescence      | Presence of viable and non-viable cells | Few viable cells visible | Presence of viable and non-viable cells | Presence of viable and non-viable cells |
| observations      |        |      |       |         |
| Synthetic medium  |        |      |       |         |
| cultures          |        |      |       |         |
| Inhibition rate   | 92%    | 100% | 75%   | –       |

For the second run, the same system was used. Treatment durations were shorter and equal to 1 and 5 minutes (E_{dens} = 90 and 450 J/cm²). Five gas mixtures were tested. Figure 8-a represents the CFU number obtained for each gas mixture and treatment duration as compared to the untreated reference. For 1 minute treatments, whatever the oxygen content in the gas mixture, no differences can be observed between the treated samples and the references. However, it seems that after a 5 minute treatment and for gas mixtures containing 1, 5 or 10 % of oxygen, there is a partial inhibition of fungi.

The third run was conducted in the same conditions but for treatment duration and an energy density equal, respectively, to 20 minutes and 1800 J/cm². It can be seen on figure 8-b that the treatment conducted with 5% of oxygen seems again to be the most efficient.

![Figure 8](image)

Figure 8. Results of synthetic medium culture obtained for the (a) second and (b) third run.

The fourth run was conducted with the custom-made reactor driven by the pulsed HV generator for the same gas mixtures conditions. Treatment durations were equal to 3 and 20 minutes and, consequently, energy densities were around 1800 and 12000 J/cm². The results presented on figure 9 seem to show that 3 minutes of treatment are not sufficient in those conditions to inhibit the fungi growth. Nevertheless, after 20 minutes of treatment, for gas mixtures containing 0 and 5 percent of oxygen a partial inhibition can be observed.

8. Discussion

It can be seen on figures 8 and 9 that the error bars are quite large. In fact, there is a low reproducibility which might be due to a problem encountered during slide preparation. The liquid culture of fungi is not homogeneous because of the presence of spores and hyphae. As a result, the quantity of fungus deposited on the slides is difficult to control. The protocol is now being improved in order to avoid this problem.
However, the results show some tendencies. It seems that treatments conducted with a gas mixture containing 5% of oxygen would be the most efficient and that a minimum of 5 minutes of treatment is required to observe a partial inhibition of fungi.

The electrical study of the two systems showed that the injected energy densities are higher for the second one for an equivalent time of treatment. Nevertheless, for the moment no distinction can be made concerning the efficiency of one system to the other regarding to fungi treatment.

Ozone measurements revealed that ozone production is different for the two systems and also that optimal treatments are not necessarily obtained in conditions where ozone densities are the highest, denoting that other species play also a role in fungi treatment.

9. Conclusion and perspectives
To conclude, it can be said that a new curative fungi treatment, conducted in afterglow conditions at atmospheric pressure has been developed. Some of the first results are really encouraging, but the protocol has to be improved in order to draw reliable conclusions.

The influence of other parameters has to be studied and some other characterisation means could be considered in order to go further in the study. For example, scanning electron microscopy could give information about the effect of the afterglow on the fungi at the cell scale. The afterglows have also to be analysed more precisely in order to determine the present species and to try to understand the mechanisms involved during the treatment.

Acknowledgements
This work is financially supported by the French National Research Program ANR-Plasmapal and by a French National Partnership (CIFRE convention: ANRT-Beynel Manustock Society-LEGP).

References
[1] Moreau S, Moisan M, Tabrizian M, Barbeau J, Pelletier J, Ricard A and Yahia L’H 2000 J.Appl. Phys. 88 1166
[2] Moisan M, Barbeau J, Moreau S, Pelletier J, Tabrizian M and Yahia L’H 2001 Int. J. Pharm. 226 1–21
[3] Laroussi M and Leipold F 2004 Int. J. Mass. Spectrom. 233 81–6
[4] Panousis E, Clement F, Loiseau J-F, Spyrou N, Held B, Larrieu J, Lecoq E and Guimon C 2007 Surf. Coat. Technol. 201 7292–302
[5] Panousis E, Clement F, Lecoq E, Loiseau J-F and Held B 2008 Experimental investigation of an atmospheric pressure Dielectric Barrier Discharge (DBD) under bi-polar pulsed high voltage excitation Proc. GD08 Cardiff
[6] Jones K H and Senft J A 1985 J. Histochem. Cytochem. 33 77–9