# Investigations of bacterial inactivation and DNA fragmentation induced by flowing humid argon post-discharge

**S. Limam[1], M.J. Kirkpatrick[1], B. Dodet[1], S. Salamitou[2], M.S. DuBow[2], E. Odic[1]**
[1]SUPELEC - E3S Département d'Electrotechnique et de Systèmes d'Energie, F-91192 Gif-sur-Yvette Cedex, France
[2]Institut de Génétique et Microbiologie, Université Paris-Sud 11, F-91405 Orsay, France
**e-mail: emmanuel.odic@supelec.fr**

**Résumé**

Bio-contaminated surfaces were exposed to an atmospheric flowing post-discharge, i.e. without direct contact of the plasma with the surface. The non-thermal plasma source was a dielectric barrier discharge in a cylinder-to-cylinder geometry. Using humid argon as a feed gas, a reduction of 6 orders of magnitude of survivors could be obtained for *Escherichia coli*. An investigation of the bacterial inactivation mechanisms during the plasma induced treatment was conducted. For this purpose, DNA (genomic DNA in aqueous solution) degradation by the plasma process was studied, assuming that the bacterial inactivation is obtained when the bacterial DNA is fragmented. According to the operating conditions (feed gas flow rate and discharge input power), DNA fragmentation was evaluated in correlation with aqueous phase hydrogen peroxide concentration measurements. It appears that hydrogen peroxide is not the only responsible factor for DNA fragmentation.

## Introduction

Non-thermal plasma technologies have been heavily investigated during the last decade for surface decontamination of thermally sensitive materials. Several techniques were studied with different excitation sources operating in different gases from 1 Torr to atmospheric pressure [1-4]. The dielectric barrier discharge (DBD) process under investigation operates at atmospheric pressure in humid argon. The contaminated surfaces to be treated are exposed to the flowing post-discharge, i.e. to the discharge products, without direct contact of the plasma with the surface. Assuming that the bacterial inactivation is obtained when DNA damage occurs, investigations of a model planktonic microorganism inactivation and DNA molecule fragmentation were conducted during plasma treatment in separate experiments.

## Experimental

For all experiments, the DBD reactor (Fig.1) consisted of a stainless steel rod (2 mm diameter) centered in a dielectric tube (6 mm and 3 mm external and internal diameters respectively) externally covered with a 24 mm length copper tape connected to ground. The inner electrode was connected to an AC (30kHz) high voltage power supply (2.5-10W discharge input power). The DBD reactor was fed with humid argon (RH ≥ 95% at room temperature, 0.5-2L/min). The outlet of the discharge tube was connected to the treatment vessel in which biological samples were exposed to the flowing post-discharge. More precisely, biological samples were placed 5 mm from the tube outlet. The bacteria were treated in 10µL (20%Vol. LB in distilled water) droplets (~$10^8$ bacteria/mL *Escherichia coli* DH10B strain) spotted on sterile glass slides. Once plasma treatment achieved, bacterial inactivation was evaluated by direct colony counting after collection and incubation (24h at 37°C).

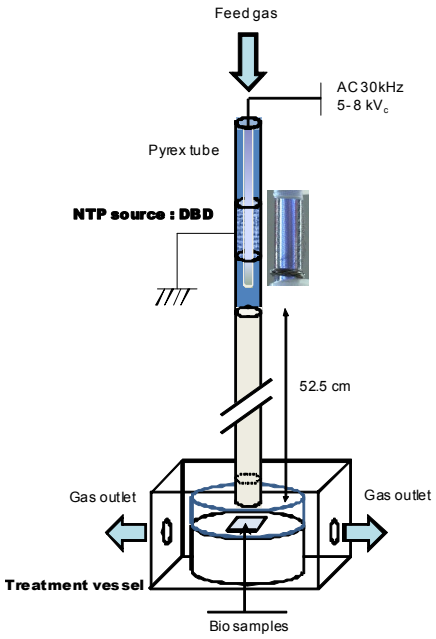

Fig. 1: DBD source and bio sample treatment device

DNA solutions were treated in 10 µL distilled water droplets (1mg/mL) spotted on sterile glass slides. As a model DNA, genomic DNA (salmon sperm) was used. After treatment, agarose gel electrophoresis was used to separate DNA molecules according to their molecular weight and 3-D structure.

Hydrogen peroxide is, with oxygen and hydrogen, an end product of water dissociation by excited argon atoms. Its high Henry's Law constant ($10^5$ M/atm) and oxidative properties lead to a possible

decontamination effect. Hydrogen peroxide concentration was measured in 10 µL distilled water droplets submitted to the plasma post-discharge, using a colorimetric method [2].

## Results

After a 10 min exposure to the DBD post-discharge (2.5W - 0.5L/min humid argon) of 10 µL water droplets, 10 µL bacteria suspension droplets and 10 µL DNA solution droplets, the following results were respectively obtained: (i) 150ppm (w/w) hydrogen peroxide concentration, (ii) reduction of 3 orders of magnitude of survivors (from $10^7$ to $10^4$ *E. coli*), (iii) fragmentation of the genomic DNA (Fig. 2: lanes 1&2 to be compared to the control in lane 3). The same quantity of DNA was incubated during 10 min in $H_2O_2$ solutions with increasing concentrations from 100ppm to 250ppm (lanes 10, 12, 14, 16 in Fig.2). No DNA fragmentation was observed, demonstrating the low activity of hydrogen peroxide for DNA fragmentation and the role played by other oxidative species, e.g. OH and/or $HO_2$. Note also that an increase in discharge input power led to a higher DNA fragmentation (see migration band enlarged and shifted toward low molecular weight – bottom of Fig. 2 lanes 4,5,7,8).

In order to reduce the transfer time of short lived species from the DBD source to the surface to be treated, the total flow rate was increased and in the case of some bacterial inactivation experiments, a 525mm PFA tube (6mm ID) could be inserted between the outlet of the DBD tube and the biological samples, in order to estimate the decontamination efficiency loss according to transfer time of the activated species from the plasma source. Results are presented in Fig.3 for *E coli* inactivation. A reduction of at least five orders of magnitude was achieved within 10 min.

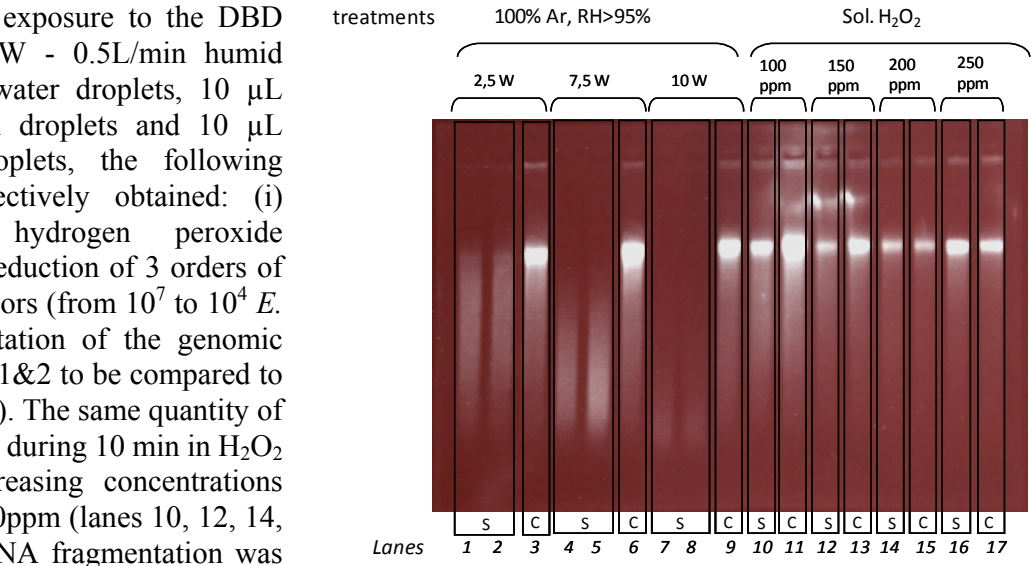


Fig. 2: Genomic DNA agarose gel electrophoresis after different treatment conditions. Lanes 1 to 9: 10 min. plasma treatment (2.5W - 0.5L/min humid argon). Lanes 10 to 17: 10 min incubation in $H_2O_2$ sol. (S = samples, C = controls, migration from the top to the bottom, .i.e. from high molecular weight to low molecular weight)

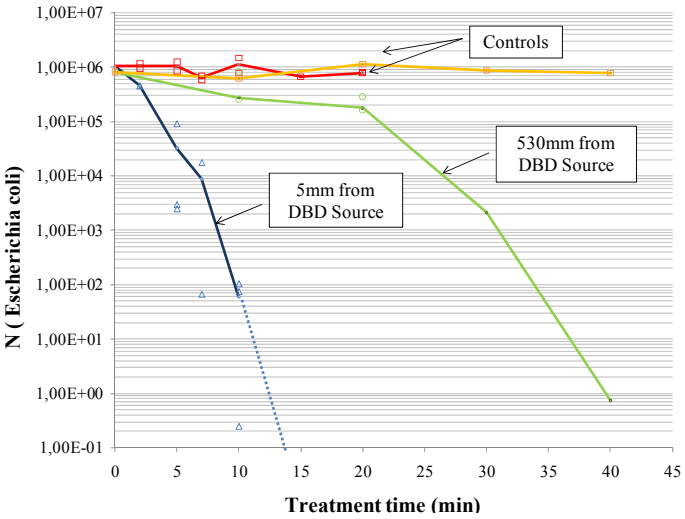


Fig.3: *E. coli* survivors vs. exposure duration to DBD flowing post-discharge (2.5W - 2L/min humid argon)

## References


[1] M. Laroussi, IEEE Trans. Plasma Sci. **30** (2002) 1409.
[2] M.K. Boudam, B. Saoudi, M. Moisan, A. Ricard, J. Phys. D: Appl. Phys. **40** (2007) 1694.
[3] M.J. Kirkpatrick, B. Dodet, E. Odic, Int. J. Plasma Environ. Science Technol. **1** (2007) 96.
[4] J.-P. Sarrette, S. Cousty, N. Merbahi, A. Nègre-Salvayre, F. Clément, Eur. Phys. J. Appl. Phys. **49** (2010) 13108.