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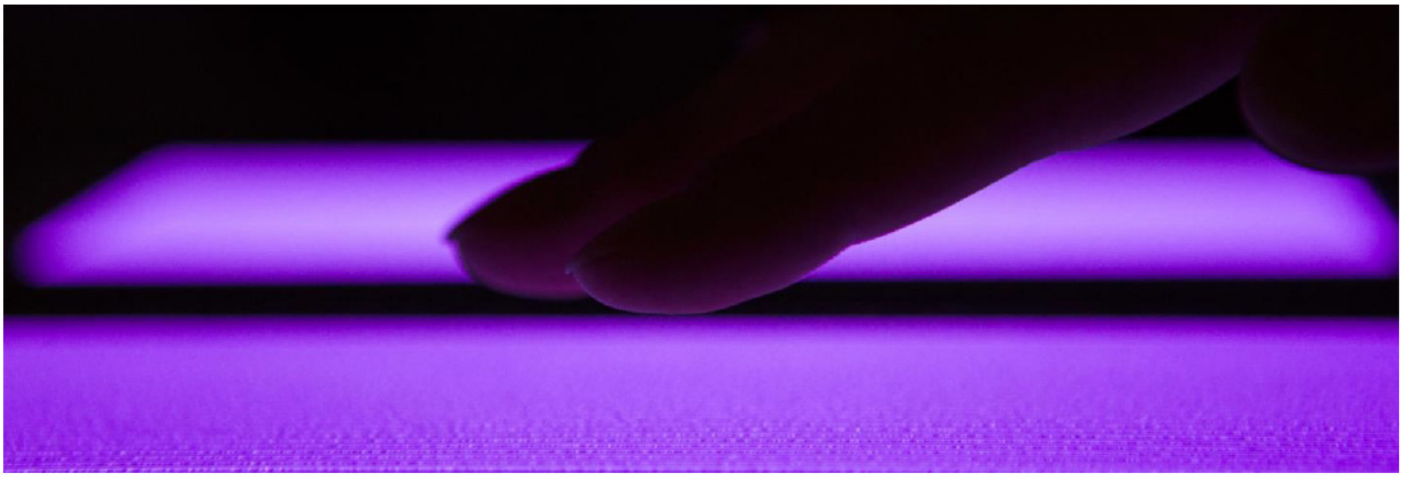
# International Symposium on High Pressure Low Temperature Plasma Chemistry

**with joint COST TD1208 workshop Non-Equilibrium Plasmas with Liquids for Water and Surface Treatments**



# Book of Contributed Papers

**Masaryk University**

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**Non-Equilibrium Plasmas with Liquids**

**for Water and Surface Treatment**

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**Contact Address**
Department of Physical Electronics
Faculty of Science
Masaryk University
Kotlarska 2
611 37 Brno
Czech Republic
Phone: +420 549 498 239
E-mail: hoder@physics.muni.cz
http://www.physics.muni.cz/kfe/

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# FORMATION OF RONS IN COLD AIR PLASMA ACTIVATED WATER AND THEIR EFFECTS ON CELL MEMBRANES OF *ESCHERICHIA COLI*

B. Tarabová[1], E. Doležalová[2], P. Lukeš[2], Z. Machala[1]
[1]*Faculty of Mathematics, Physics and Informatics, Comenius University in Bratislava, Mlynská dolina, Bratislava, Slovakia*
[2]*Institute of Plasma Physics of the CAS, v.v.i., Za Slovankou 3, Prague, Czech Republic*

E-mail: tarabova@fmph.uniba.sk

In this work, we focused on the detection of secondary reactive oxygen and nitrogen species (RONS) formed by cold plasma induced gas-liquid chemistry in air discharge activated water and liquids. Plasma induced chemical changes in plasma activated water (PAW) were correlated with the antibacterial effect, sublethal injury of bacterial cells and peroxidation of membrane lipids. The contribution of the electric field induced by the plasma treatment was evaluated by the detection of membrane permeabilization. The bactericidal effects of direct (plasma and PAW) and indirect (only PAW) treatments were compared with long-term preservation of the PAW with its antibacterial properties.

**Keywords**: cold air plasma; plasma activated water; sublethal injury; lipoperoxidation

## 1 Introduction

Nowadays, cold atmospheric pressure plasmas generated by electrical discharges in gas-liquid interface or directly in water are studied. Plasmas generated in air and in contact with liquids generate a number of primary reactive species in the gas phase, which induce formation of secondary reactive species in the liquid phase through the gas-liquid interface. Reactive oxygen and nitrogen species (RONS) such as hydrogen peroxide, hydroxyl radical, superoxide, nitrites/nitrates, hypochlorites and peroxynitrites induce chemical changes in water solutions and the plasma activated water (PAW) and liquids are created. Thanks to the synergistic effects of the plasma agents (electric field, electrons and ions, RONS) and the induced chemical changes in the liquid, cold air plasmas and PAW lead to various biocidal effects on microorganisms and cytotoxic or growth stimuli effect on cells [1-2]. The investigation of the effects on cells induced by plasma agents or PAW is very important for further bio-medical applications. Therefore the aim of our work was to detect the chemical changes induced via the formation of RONS in air plasma activated liquids and their effects on bacterial cells and cell membranes.

## 2 Experimental set-up and methods

### 2.1 Transient spark with electrospray

Transient spark (TS) discharge was generated in ambient air at atmospheric pressure in point-to-plane geometry (Fig. 1). TS is a self-pulsing repetitive streamer-to-spark discharge with the spark current pulse (20-30 A) duration shorter than 100 ns. Due to the short pulse duration, the plasma cannot reach the thermal equilibrium conditions and remains at low gas temperature. A positive high voltage (10-12 kV) was applied on the hollow needle electrode. The water solutions/bacterial suspensions were pushed through the high voltage electrode by a syringe pump. This allowed the water solutions to flow directly through the active zone of the discharge. Furthermore, due to the applied high voltage, we observed the effect of the electrospray. This electrospraying enhanced the mass transfer of reactive oxygen and nitrogen species into the treated liquid solutions [3].

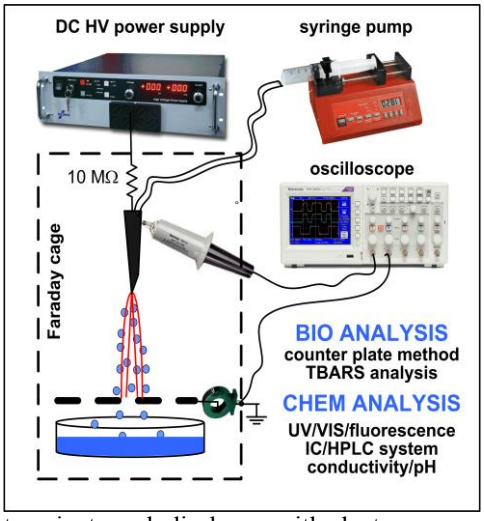


**Fig. 1.** Experimental set-up of the transient spark discharge with electrospray.

## 2.2 Chemical methods

Aqueous solutions with different initial pH and electrolytic conductivities σ were used for plasma treatment by the dissolution of different salts in deionized water: water ($NaH_2PO_4$ solution), phosphate buffer ($NaH_2PO_4/KH_2PO_4$ solution), saline solution (0.85% NaCl) and phosphate buffered saline. RONS in plasma treated water (plasma activated water PAW) were detected mostly by colorimetric methods (UV/VIS absorption spectrometer UV-1700 SHIMADZU) or fluorescent spectroscopy:

1. Analysis of $H_2O_2$: Titanyl ions $Ti^{4+}$ react with $H_2O_2$ in the presence of $NaN_3$ and create pertitanic acid with the absorption maximum at 407 nm.
2. Analysis of $NO_2^-$: Nitrites $NO_2^-$ react with Griess reagents and create azo-dye with the absorption maximum at 540 nm (Nitrate/Nitrite Colorimetric Assay Kit, Cayman Chemical).
3. For qualitative detection of peroxynitrites was used the fluorescent dye $H_2$DCFDA (2,7-dichlordihydrofluoresceine diacetate).
4. Superoxide $O_2^{\cdot-}$ was detected indirectly as the increase of $H_2O_2$ concentration after superoxide dismutation by the superoxide dismutase enzyme.

## 2.3 Microbial handling

The antibacterial effect was tested on suspensions of planktonic form of *Escherichia coli* ATCC 25922. The number of living microbial was evaluated by classical thermostatic cultivation on Petri dishes. Furthermore, the effects of the chemical changes of PAW on the bacterial cells were detected – sublethal injury of outer and cytoplasmic membrane, the peroxidation of membrane lipids, the loss of membrane integrity and the metabolic activity [4-5]. To study the presence of sublethally injured cells, samples were plated on two selective media: TSAYE (tryptone soya agar enriched with yeast extract) with 3.1% of sodium chloride (TSAYE-SC) and TSAYE with 0.3% of bile salts (TSAYE-BS). Cells become sensitized and unable to grow on the selective media containing bile salts if their outer membranes are damaged, whether the loss of tolerance to the presence of sodium chloride is linked with the loss of the functionality and/or integrity of cytoplasmic membrane. To evaluate the cell permeabilization, we tested the fluorescent staining - propidium iodide or DAPI (4'6-diamino-2-phenylindole). Reactive species forming during the plasma treatment in PAW cause the peroxidation of cell membrane lipids, which was detected by the thiobarbituric acid test (TBA). Thiobarbituric acid reacts with secondary product of lipid peroxidation – malondialdehyde forming the pink colored MDA-$TBA_2$ complex.

## 3 Preliminary results

The transient spark discharge was generated in ambient air at atmospheric pressure in the direct contact with treated liquid solutions. Changes of pH, conductivity and formation of RONS were observed in water or PB solutions. In non-buffered water solution was observed the decrease of pH (5 → 3.2) instead of PB buffered solution, where pH remained at 6.9. Dissolution of $NO_x$ along with the formation of $NO_2^-$, $NO_3^-$ was responsible for the acidification of the plasma treated solution. Measured concentration of long-lived species such as $H_2O_2$, $NO_2^-$ and $NO_3^-$ in PAW on post-treatment time showed the ongoing post-discharge chemistry – acidic decomposition of nitrites and decreasing concentrations of $H_2O_2$ and $NO_2^-$ because of the peroxynitrite formation. The higher bactericidal effect (Fig. 2a) was observed in non-buffered solutions and it was linked with the different RONS chemistry associated with acidification (pH ~ 3). At acidic pH radicals like $OH^{\cdot}$, $NO^{\cdot}$ and $NO_2^{\cdot}$ are formed by acidic decomposition of nitrites and also peroxynitrites, which posses a strong cytotoxic effects on cells. Bactericidal effect of PAW remained for some time post plasma treatment, as shown in Fig. 2b, where indirect treatment means 10 min incubation of PAW with bacteria. Furthermore, the longer incubation times were tested and the preservation of bactericidal effect of PAW by long-term storage by deep freezing was investigated.

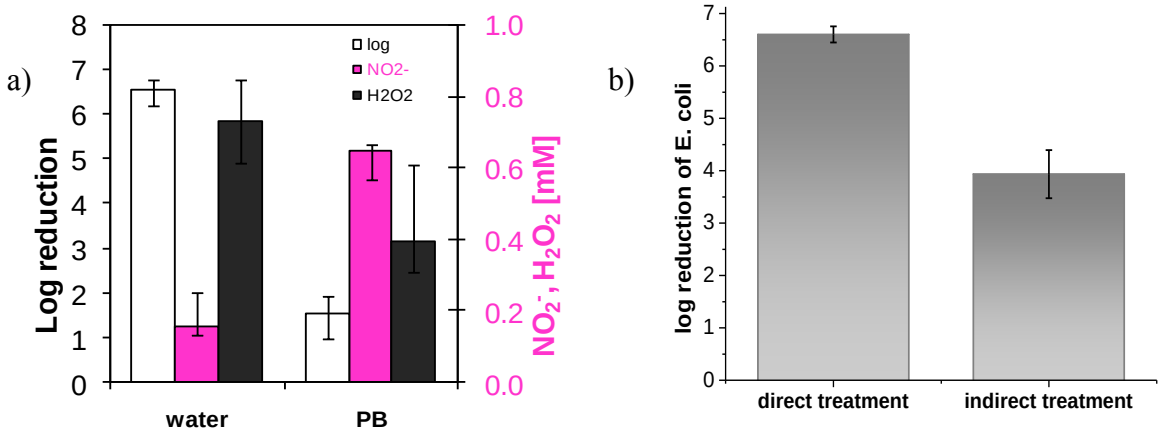


**Fig. 2.** a) Bactericidal effect of direct treatment of E. coli suspension linked with the nitrites and hydrogen peroxide concentrations. b) Comparison of bactericidal effect of direct plasma treatment and indirect PAW treatment (10 min incubation).

Reactive species (mostly $OH^{\cdot}$, $NO^{\cdot}$, $NO_2^{\cdot}$ and $O_2^{\cdot -}$) forming during the plasma treatment in PAW are responsible for the oxidative damage of the cell membrane due to the peroxidation of membrane lipids. The significant increase of the MDA-$TBA_2$ complex was measured on bacteria in non-buffered solutions after plasma treatment. Due to the minimal amount of detected sublethally injured bacterial cells in buffered solutions, we found out that the sublethal injury was also accompanied with the acidification and formation of the cytotoxic radicals in PAW.

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**References**

1. M. Laroussi, IEEE Trans. Plasma Sci. 30, 1409-1415 (2002)
2. S. Bekeschus et al., Oxid. Med. Cell. Longev. 2016, 5910695 (2016)
3. Z. Machala et al., Plasma Process. Polym. 10, 649-659 (2013)
4. C. Arroyo et al., Lett. Appl. Microbiol. 51, 525-531 (2010)
5. E. Doležalová et al., J. Phys. D: Appl. Phys. 49, 074501 (2016)