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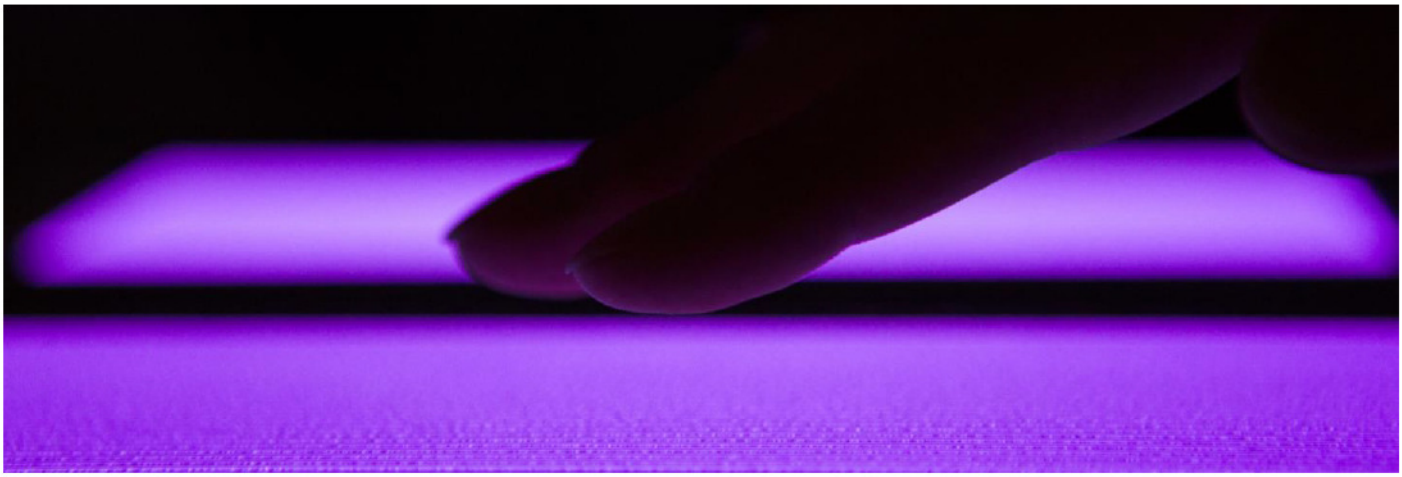
with joint COST TD1208 workshop Non-Equilibrium Plasmas with Liquids for Water and Surface Treatments



Book of Contributed Papers

Masaryk University

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Non-Equilibrium Plasmas with Liquids

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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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IDENTIFICATION OF THE REACTIVE SPECIES PRODUCED BY TRANSIENT SPARK DISCHARGE IN GAS AND LIQUID PHASE AND ITS EFFECT ON *ESCHERICHIA COLI*

K. Kučerová, M. Janda, K. Hensel
Faculty of Mathematics, Physics and Informatics, Comenius University, Bratislava, Slovakia

E-mail: katarina.kucerova@fmph.uniba.sk

We used the self pulsing transient spark discharge driven by DC power supply at atmospheric pressure above the circulating water solution in various gas mixtures of nitrogen and oxygen. For better understanding the plasma induced chemical and biological changes in solutions we analyze both the gas and the liquid phase. We detected NO, NO_2, O_3 molecules in the gas phase by FTIR, while in the liquid phase we measured H_2O_2, NO_2^- and $^{\bullet}OH$ radical concentrations by UV-VIS and fluorescence spectroscopy. The results from gas and water analysis were put in a correlation with the bactericidal effect of TS on *E. coli* in water solution.

Keywords: transient spark discharge; reactive oxygen and nitrogen species; bacteria

1 Introduction

The electrical discharges generated in gasses at atmospheric pressure are the source of low temperature plasma. Their properties predetermine them for many industrial, environmental and even biomedical applications. Low temperature plasma generated in air like mixtures in contact with water induces numerous reactive species in the gas phase, which could subsequently diffuse into liquid phase and induce further chemical reactions. These chemical changes in the liquid also affect the biological matter inside the treated liquid. There is number of publications dedicated to research of chemical changes in plasma treated solutions also studying the effects of the plasma on bacteria, eukaryotic cells and tissues [1-5]. Although a great research effort has been dedicated to elucidate the role of the plasma interaction with biological matters, the exact mechanisms of their interaction are still not enough clear. Therefore, we investigate the transient spark (TS) discharge generated in various gas mixtures of nitrogen and oxygen in contact with water solutions. We separately explore the products generated in the gas phase and in the liquid phase and relate these results with bactericidal effects on *E. coli*.

2 Experimental

In Figure 1 is schematically depicted the principle of water electrode system (side view). The transient spark (TS) discharge was generated in point to plane geometry (inter-electrode distance 1 cm) driven by DC high voltage of positive polarity [6]. Downstream, the grounded electrode, through the discharge zone a liquid was repetitively circulated (flow rate 14 mL/min). The discharge system was enclosed in a small chamber (volume 12.5 mL) to allow variation of O_2/N_2 gas mixtures (flow rate 2 L/min). As a liquid, we used 2 mM phosphate buffer (**PB**, 550 µS/cm, pH 7) and its non-buffered counterpart the solution of monosodium dihydrogen phosphate $NaH_2PO_4.2H_2O$ (**W,** "water", 600 µS/cm, pH 5) to study the pH dependent changes. In these solutions were Gram-positive bacteria *Escherichia coli*

were dissolved with initial concentration ~ 10^7 CFU/mL. The bacterial inactivation was evaluated by standard colony counting method. The chemical species produced in the gas phase by the TS discharge were measured by FTIR absorption spectroscopy (*IRAffinity-1S, Shimadzu*). In the liquid phase we measured the concentration of hydroxyl radical ($^{\bullet}$OH) by fluorescence spectroscopy using terephthalic acid as a probe. For the analysis of the other reactive species in water solution we used UV-VIS absorption spectroscopy (*UV 1800, Shimadzu*). The H_2O_2 concentration in the liquid was evaluated by titanyl ions from titanium oxysulfate ($TiOSO_4$) forming yellow-colored complex. The NO_2^- and NO_3^- were measured by commercial kit (*Cayman*) based on reaction with Griess reagents.

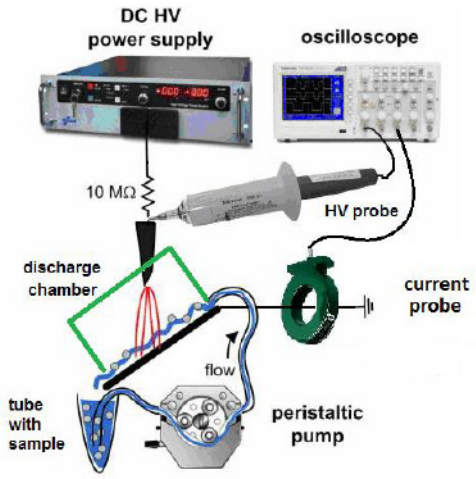

Fig. 1. Scheme of the experimental setup.

3 Results and Discussion

We present the results of self-pulsing TS discharge effect on buffered (PB) and non-buffered (W) water solutions containing bacteria. The TS discharge was generated in various O_2/N_2 gas mixtures. The typical amplitude of the applied voltage was U_{max} = 10 – 13 kV, amplitude and frequency of the discharge current pulses were I_{max} = 5 – 18 A and f = 1.5 – 4 kHz, respectively. The character of the TS discharge was slightly different and depended on the ratio of O_2/N_2 gas mixtures; however the total mean power was nearly the same ~5-7 W.

The reactive molecules in the gas phase and the subsequent effect on reactive chemical species produces in water and effect on bacteria were observed. We detected NO and NO_2 molecules in gas mixtures containing both O_2 and N_2 molecules by FTIR measurements. The NO and NO_2 concentrations were increasing with O_2 ratio up to 50%, where the concentration of NO and NO_2 achieved maximum, 264 ppm and 60 ppm respectively. In pure O_2 we detected only O_3 molecules (~ 110 ppm).

The concentrations of H_2O_2, NO_2^- and $^{\bullet}$OH radical in solutions depended on O_2/N_2 ratio in gas mixture. In the case of mixtures containing both O_2 and N_2, the most significant pH decrease and the relatively high concentration of reactive species (0.4-0.5 mM) was observed compared to pure N_2 and O_2. When the discharge was generated in pure O_2, the NO_2^- concentration was negligible, but the concentration of H_2O_2 was relatively high due to higher formation of $^{\bullet}$OH radicals in the gas phase that subsequently forms and accumulates H_2O_2 in water solution and also due to lack of NO_2^- that could potentially react with it. In pure N_2 the discharge produced only a small amount of NO_2^- because of lack of O_2, therefore only a small decrease of pH was observed.

The gas composition above the treated water solution affected the production of reactive species in liquid solutions and subsequently also inactivation of bacteria. Even the differences among respective gas mixture were not significant, the bacterial inactivation rate expressed as a logarithmic reduction was highest in air like mixture ~ 2.5 log reduction. In gas mixtures where O_2 and N_2 are present in relatively equal rate, also H_2O_2 and NO_2^- are present equally in solution. Synergetic effect of H_2O_2 and NO_2^- in acidic conditions can lead to formation of peroxinitrites ($ONOO^-$) and is responsible for overall strong bactericidal effect [5]. The difference between the effect in buffered (PB) and non-buffered (W) solution can be seen in the Fig. 2. which shows that bacterial inactivation is more efficient in W solution, where pH decrease and conductivity increase were more pronounced than in PB.

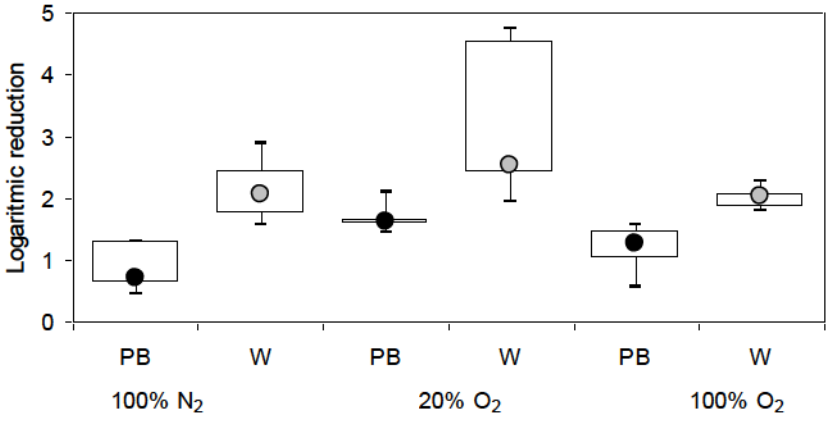

Fig. 2. The logarithmic reduction of *E. coli* in phosphate buffer (PB) and "water" (W) 5 min treated by TS discharge generated in various gas mixtures (100% N_2, 20% O_2, 100% O_2), data shown as maximum, upper quartile, median, lower quartile, minimum, from 5 independent measurement.

4 Conclusion

The low temperature plasma generated by the TS discharge at atmospheric pressure were generated in various O_2/N_2 gas mixtures in contact with water solutions in order to explore the relationship between reactive species generated in the gas and liquid phase and subsequent effect on bacteria *E. coli*. FTIR spectroscopy dominantly showed NO and NO_2 molecules in most of the gas mixtures, while O_3 was observed only in pure O_2. In the liquid phase H_2O_2, NO_2^- and $^{\bullet}OH$ reactive species were detected. The *E. coli* inactivation was more pronounced in mixtures in non-buffered solution. In conclusion, for the efficient *E. coli* inactivation both H_2O_2 and NO_2^- need to be present in the treated water solution.

Acknowledgment

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