Bioplasmas and Plasmas with Liquids

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BOOK OF ABSTRACTS

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Investigation of the detection methods of reactive oxygen and nitrogen species in air plasmas activated water

B. Tarabová[1], P. Lukeš[2], M. Hammer[3], K. Kučerová[1], T. von Woedtke[3], S. Reuter[3], K. Hensel[1], Z. Machala[1]

[1]*Comenius University in Bratislava, Mlynská dolina, 84248, Bratislava, Slovakia*
[2]*Institute of Plasma Physics, AS CR v.v.i., Za Slovankou 3, 18200, Prague, Czech Republic*
[3]*INP Greifswald e.v., Felix-Hausdorff-Str. 2, 17489, Greifswald, Germany*

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 H_2O_2, hydroxyl radical $OH^{\cdot}$, nitrites NO_2^-, nitrates NO_3^-, hypochlorites OCl^- and peroxynitrites $ONOO^-$ induce chemical changes in water solutions and leads to various biocidal effects on microorganisms or therapeutical effects for biomedical applications. It was shown that the acidic environment with plasma agents (especially hydrogen peroxide, nitrites, nitrates) lead to the strong bacterial inactivation [1]. The detection of reactive species in the plasma activated water (PAW) is challenging due to the presence of highly reactive species and possible cross-reactivities. Therefore it is crucial to either develop new methods of their detection or adapt the methods known from analytical chemistry and biology for special conditions in PAW.

In this work we focused on the detection of RONS formation induced by plasma gas-liquid chemistry in PAW. Hydrogen peroxide, nitrites, nitrites, peroxynitrites and dissolved ozone were detected in solutions electrosprayed directly through the DC-driven air transient spark discharge. Water solutions with different initial pH and conductivities were differentiated according their buffering capacity, pH or content of chlorine.

Concentrations of measured hydrogen peroxide, nitrites and nitrates by ion chromatography or colorimetric method were correlated with the reduction of *E. coli* population. The acidic pH is important for the following processes responsible for bacterial inactivation and the post-discharge antimicrobial effect of PAW: acidic decomposition of nitrites, formation of peroxynitrites and their acidic decay. Furthermore, we tested the accuracy of the colorimetric Griess assay for nitrites detection by comparison with high precision ion chromatography. In addition, to avoid possible interference of hydrogen peroxide on the Griess assay, we attempted to increase the detection limit of the assay using the enzyme catalase as a H_2O_2 scavenger. The comparison of tested methods showed no difference in NO_2^- concentration and by the addition of catalase we showed that H_2O_2 does not have any influence of the Griess reagent assay.

The presence of dissolved ozone in PAW was primarily detected by standardized colorimetric indigo method. Nevertheless, indigo was strongly decolorized without any difference in PAW and in chemically simulated plasma treated solution with similar chemical composition and pH as PAW, where no ozone could have been present. Furthermore, by using phenol as the chemical probe and measuring its degradation products after plasma treatment by high performance liquid chromatography we detected no muconic acid as a specific product with ozone [2]. This result supports the fact that indigo method is not selective to ozone in PAW and it seems that the hydroxyl radical as a product of peroxynitrites decay in acidic PAW is responsible for the false positive reaction of the indigo method.

We tested the possible use of 2,7-dichlorodihydrofluorescein diacetate fluorescent dye (H_2DCFDA) for peroxynitrites detection in PAW. By using several scavengers of RONS we demonstrated that at least in plasma activated solutions with no Cl^- present and therefore no hypochlorite (OCl^-) anions created, the H_2DCFDA fluorescence signal is dominantly due to $ONOO^-$ and this fluorescent spectroscopic method is promising for peroxynitrites detection in PAW under considering some limitations.

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