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Reactive species in gas and liquid phases produced by transient spark discharge in various N_2/O_2 mixtures and their effect on *Escherichia coli*

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Abstract: The self-pulsing transient spark discharge was generated in contact with water at atmospheric pressure in various nitrogen-oxygen gas mixtures. Reactive species of nitrogen and oxygen produced by the discharges in the gas and in the liquid phases were measured by means of various spectroscopic methods. Concentrations of NO, NO_2 and O_3 in the gas phase were measured by infrared spectrometry, while UV-VIS absorbance and fluorescence spectroscopy were used to measured H_2O_2, NO_2^-, NO_3^- and $^{\bullet}OH$ radical concentrations in the liquid phase. The plasma induced chemical effects were correlated with observed bactericidal effects on *Escherichia coli* with respect to the gas mixture composition.

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

1. Introduction

The cold plasma generated by various electric discharges has a great potential for disinfection and sterilization of surfaces, medical instruments, water, air, food, blood coagulation, wound healing or even selective cancer treatment [1-3]. The wide range of the cold plasma applications is due to its highly reactive environment, i.e. production of chemically active species and reactions, occurring while surrounding gas remains at ambient temperature. Generating the cold plasma in the gas leads to formation of highly reactive species and free radicals ($^{\bullet}OH$, $^{\bullet}H$, $^{\bullet}O$, $NO^{\bullet}$), which may further produce secondary species in the gas phase (O_3, NO, NO_2, HNO_3). When plasma is generated in contact with water these species can diffuse and dissolve in the water and induce formation of various reactive oxygen and nitrogen species RONS ($^{\bullet}OH$, H_2O_2, NO_3^-, NO_2^-, $ONOO^-$) through the plasma-liquid interface [4, 5]. The evaluation of concentrations of the reactive species in the gas and liquid phases is crucial for better understanding the chemical processes and subsequent effect of cold plasma on living organisms. In this work we investigated the effect of the gas mixture composition on the production of reactive species produced by transient spark discharge in the gaseous and in liquid phases and correlated the plasma induced chemistry with bactericidal effect on *Escherichia coli* used as model bacteria.

2. Experimental Setup

The schematic representation of the experimental setup is depicted in Fig. 1. The plasma was generated by the transient spark (TS) discharge in point-to-plane geometry and driven by positive DC power supply. The repetitive contact of the plasma with water was provided by peristaltic pump (*Masterflex L/S*) that circulated the water solution on the grounded plane electrode above which the high voltage electrode was placed (gap distance ~ 1 cm) [6]. The system was enclosed in a small chamber (volume ~ 20 cm^3) that allowed varying gas mixture composition. The electrical parameters of the TS were monitored by high voltage probe and Rogowski type current probe connected to the oscilloscope (*Tektronix TDS 2024*). The amount of water solution treated was 5 mL and the plasma exposure time was 5 min, equal for all tests.

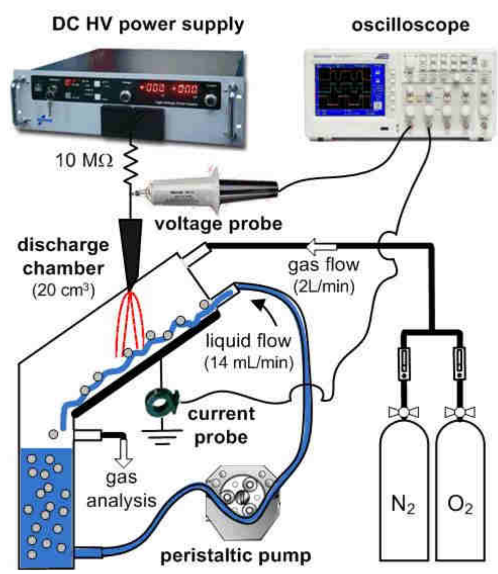

Fig. 1. Scheme of the experimental setup.

3. Materials and Methods

Several gas mixtures of nitrogen/oxygen in various ratios (0, 5, 10, 20, 50, 80 and 100 % of O_2 in N_2) and with total gas flow ~ 2 L/min, and two different water solutions, a) non buffered solution of NaH_2PO_4 (W) mimicking tap water and b) 2 mM phosphate buffer solution Na_2HPO_4/KH_2PO_4 (PB) were used. The chemical species produced in the gas phase by the TS discharge (NO, NO_2, O_3, HNO_3) were measured by FTIR absorption spectroscopy (*Shimadzu IRAffinity-1S*), while species in the liquid phase (H_2O_2, NO_2^-, NO_3^-) were measured using various colorimetric methods by UV-VIS absorption spectroscopy (*Shimadzu UV-1800*). The concentration of H_2O_2 was evaluated by its reaction with titanium ions of $TiOSO_4$ that yields in yellow coloured compound (λ = 407 nm). The concentrations of NO_2^- and NO_3^- were determined using commercial kit (*Cayman Chemicals*) based on reaction with Griess reagents (λ = 540 nm). For the measurement of $^{\bullet}OH$ radicals terephthalic acid (TA) was employed as highly selective $^{\bullet}OH$ scavenger that yields in hydroxyterephthalic acid (HTA) and can be detected by fluorescence spectroscopy (λ_{em} = 425 nm). Solution of TA was prepared by dissolving it in the distilled water with NaOH, as TA does not dissolve in acidic/neutral environment. The initial concentrations of TA and NaOH were 2 mM and 5 mM, respectively [7]. We also monitored the pH (*WTW ProfiLine pH 3110*) and the conductivity (*Greisinger GMH 3430*) of the solutions before and after plasma treatment. The biological effect of the chemical species generated by the plasma was investigated on Gram-negative bacteria *Escherichia coli* (CCM3954). Bacteria were suspended in solution with initial concentration ~ 10^7 CFU/mL and their inactivation was estimated with standard colony counting method and evaluated as a logarithmic reduction of bacterial population.

4. Results and Discussion

The TS discharge was generated in various gas mixtures of O_2 and N_2, in the contact with two different types of water solutions (W or PB). The amplitude of the DC applied voltage varied in the interval of ~ 10 – 13 kV, the amplitude of the current pulses ~ 5 – 18 A, the frequency of pulses were ~ 1.5 – 4 kHz depending of the gas mixture. The typical mean power of the TS used in this work was 1–7 W, with energy 1–7 mJ per pulse.

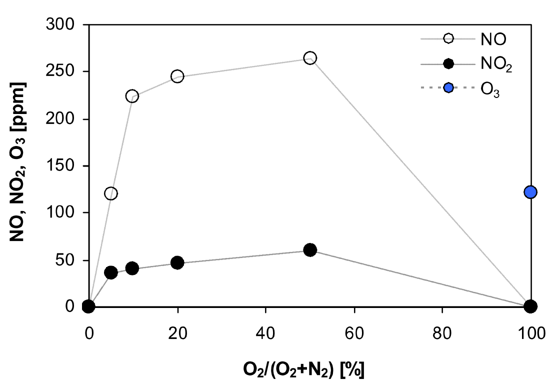

Fig. 2. Concentrations of gaseous chemical species formed by the TS discharge in N_2/O_2 mixtures.

When TS was generated in gas mixture containing both O_2 and N_2, formation of NO and NO_2 in the gas phase occurred. The NO and NO_2 concentrations increased with the increasing $O_2/[O_2+N_2]$ ratio, where the maximum concentrations of NOx was observed in the interval 20-50% $O_2/[O_2+N_2]$ (Fig. 2). In addition to NO and NO_2, small concentration of other nitrogen oxides (N_2O, N_2O_5) and acids (HNO_2, HNO_3) were observed. If the discharge was generated in pure O_2 the only product observed in the FTIR spectra was ozone O_3, while in pure N_2 no gaseous products were found in the spectra.

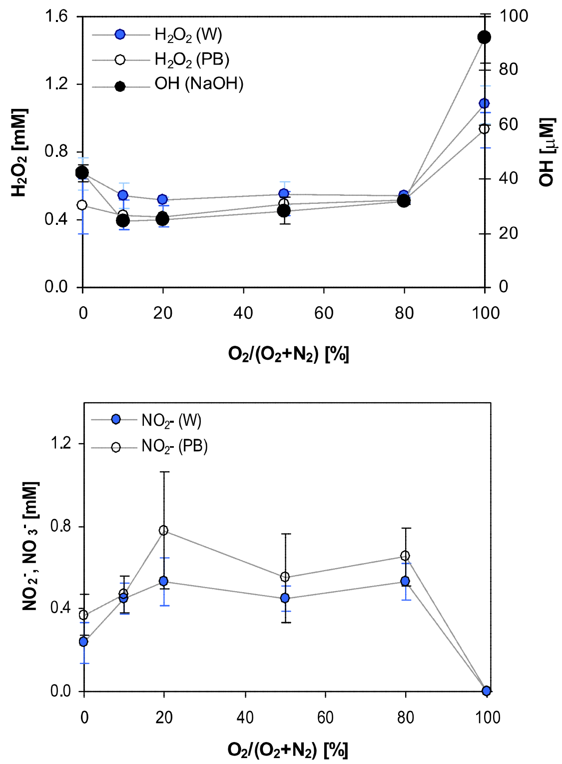

Fig. 3. Concentrations of aqueous chemical species produced by the TS discharge in N_2/O_2 mixtures.

The reactive species generated in the gas phase subsequently dissolve in the treated water solution. Therefore simultaneously with the gas analysis the concentrations of H_2O_2, NO_2^-, NO_3^- and $^{\bullet}OH$ radical in treated water solution were measured. When TS was generated in air-like mixture containing both O_2 and N_2, typical concentrations of H_2O_2 0.42-0.53 mM and NO_2^- 0.45-0.78 mM were observed depending on the $O_2/[O_2+N_2]$ ratio and water solution type (Fig. 3). For the given ratio, concentrations of H_2O_2 was usually higher in W solutions and NO_2^- was higher in PB solutions (due to neutral pH that suppress NO_2^- to be converted into NO_3^-), while concentration of NO_3^- in both solutions were similar, approx. 0.5 mM (W). The concentration of $^{\bullet}OH$ radicals as function of $O_2/[O_2+N_2]$ ratio resembles the profile of H_2O_2 concentration, that supports the idea of their involvement in the H_2O_2 production. The effect of water solution flow rate in the range of 5~20 mL/min was tested, however no significant effect on the concentration of the generated species was found. In water solution pH typically decreased from 5.5 to 3.5 and solution conductivity increased from 600 to 700 µS.cm^{-1}.

When the TS was generated in pure N_2, the concentration of NO_2^- was small and in pure O_2 it was negligible, while concentration of H_2O_2 was relatively high because of higher formation of $^{\bullet}OH$ radicals, and also due to lack of NO_2^- that could potentially react with H_2O_2. Because NO_2^- and NO_3^- are mainly responsible for acidification of plasma treated solutions, we observed only small pH decrease in solutions treated in pure N_2 and O_2.

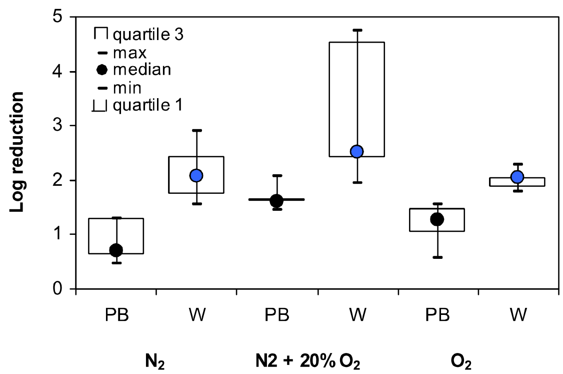

Fig. 4. Bactericidal effect of TS on *Escherichia coli.*

The chemical analyses of gas mixture and water solutions were correlated the bactericidal effect of TS on *Escherichia coli*. The decrease of bacterial population was observed for all gas mixtures. More significant decrease was observed in W solution rather than in PB solution, and in air-like mixtures rather than pure N_2 or O_2 (Fig. 4). It can be explained by the synergic effect of H_2O_2 and NO_2^- in acidic solution, which can lead to production of peroxinitrites $ONOO^-$ [4, 8]. Peroxynitrites are relatively strong oxidants with a strong bactericidal effect and they significantly contribute to the inactivation process induced by air plasma in water. On the other hand, PB solutions do not acidify enough during plasma exposure and discharges in pure N_2 or O_2 do not produce NO_2^- high enough for significant peroxinitrite chemistry and still result in some bactericidal effect, though lower than in W. So other plasma agents or chemical pathways must be involved, e.g. the effect of the electric field or UV radiation, or the effect of O_3 in pure O_2.

5. Conclusion

The cold plasma was generated by self-pulsing TS discharge at atmospheric pressure in various O_2/N_2 gas mixtures in contact with water solutions in order to understand the relationship between reactive species generated in gas and liquid phases and their subsequent effects on bacteria. In air-like mixtures the gas phase chemistry was dominated by NO and NO_2 formation. The liquid chemistry was dominated by H_2O_2, NO_2^-, NO_3^- and $^{\bullet}OH$ radical formation. The strongest bactericidal effects were found in air-like mixtures and non-buffered solutions.

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5. References

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