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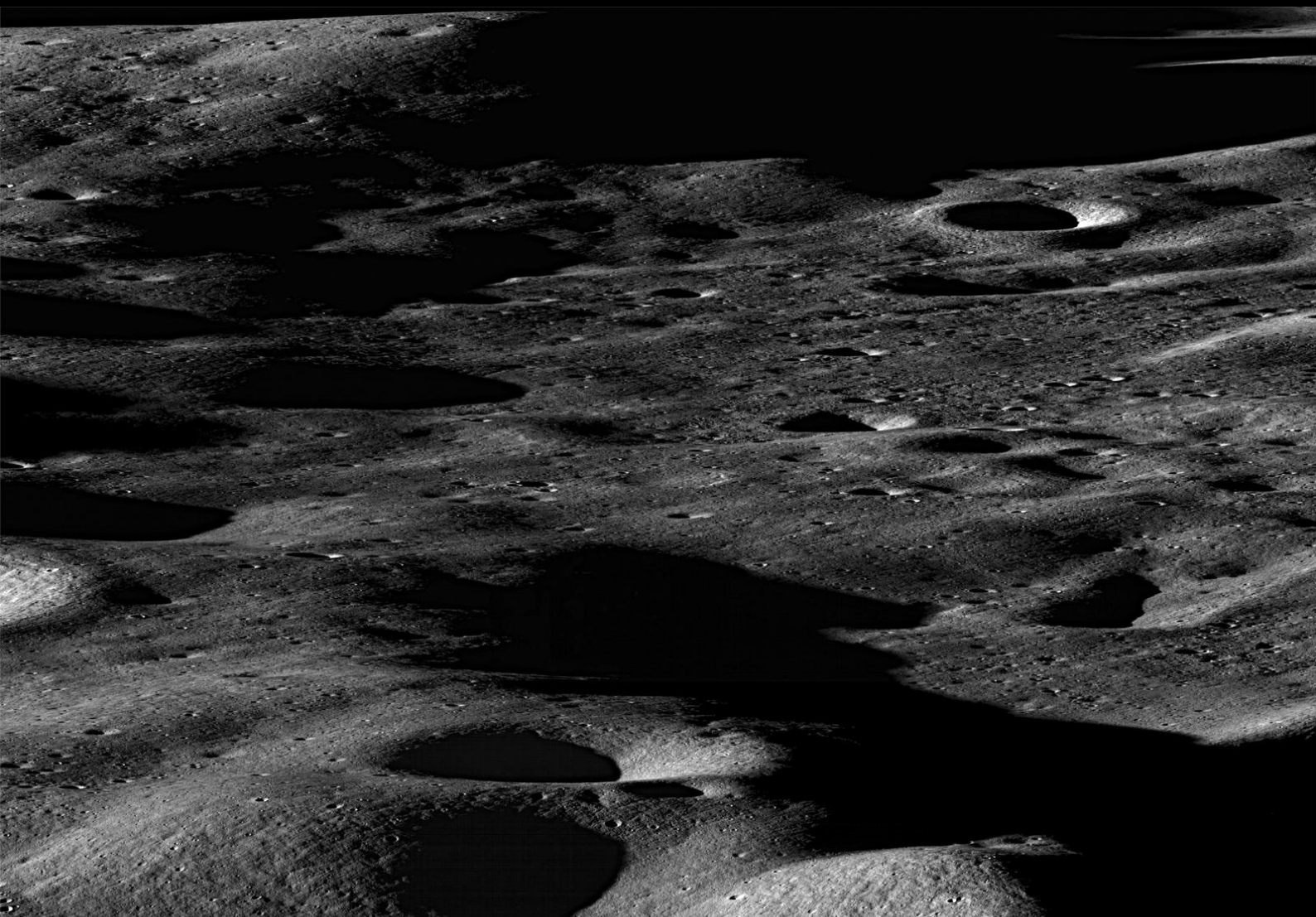

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Biological and Chemical Effect of DC Transient Spark Discharge on *Escherichia Coli*

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Abstract. We investigated the effects of cold plasma generated by DC transient spark discharge in a contact with water solutions containing *E. coli*. In the discharge system, where water solution repetitively flow through the discharge zone, we observe more pronounced chemical and biological effects in non buffered solutions compared to buffered ones. We analyzed the concentrations of various reactive species in water solutions, pH and conductivity in dependence on gas mixture composition, water solution flow rate and plasma treatment time. The chemical effects were correlated with observed bacterial inactivation.

Introduction

Plasma is a rich source of reactive species, high energy electrons and charged particles. It has the potential to be effectively used for many industrial applications including environmental or energetical ones. For biological and medical applications, the low temperature (cold) plasma is often used as it possess highly reactive electrons while surrounding gas remains at ambient temperature. The cold plasma has a great potential for sterilization of thermo sensitive materials, food and fruit treatment, blood coagulation, dental care and disinfection, tissue and wound healing, or even ulcer and tumor treatment [*Fridman* 2008; *Laroussi* 2009; *Morent* 2011; *Park* 2012]. Low temperature plasma effect strongly depends on gas composition and surroundings in which it is generated. Water based environment is natural for most of living organisms and water is also present in most living organisms. Generating the plasma in the gas in contact with water leads to formation of primary active species and radicals ($OH^{\bullet}$, $H^{\bullet}$, $O^{\bullet}$, $NO^{\bullet}$) usually via electron impact reactions with the dominant molecules in air (N_2, O_2, H_2O_2). These species may then react among themselves or with surrounding gas and form various secondary species in a gas phase (O_3, NO, NO_2, HNO_3). By the contact of plasma with water these species can dissolve into water and lead to formation of various reactive oxygen and nitrogen species RONS: dissolved ozone O_3, nitrites NO_2^-, nitrates NO_3^-, peroxynitrites $ONOO^-$. The RONS species are often considered to play the main role in the process of chemical decontamination of gas and water, as well as bacterial inactivation [*Laroussi* 2002; *Morent* 2011; *Machala* 2013, *Lukeš* 2014]. The production of nitrites and nitrates in water is probably the main reason of acidification of plasma treated solution [*Lukeš* 2012; *Machala* 2013]. The evaluation of concentration of these reactive species is critical to understand the chemical processes and subsequent biocidal effects. Despite many positive effects reported plasma interaction with cells remains still relatively not well understood. The goal and contribution of our work was to investigate the chemical effects of plasma generated in various gases and in a contact with various water solutions and correlate them with the biological effects of the plasma.

Experimental Setup

Experimental setup was designed to provide repetitive contact of water solution with discharge (Fig. 1) [*Hensel* 2015]. Water solution was driven by peristaltic pump down the grounded electrode (water electrode) above which a high voltage electrode was placed. The distance between the electrodes was 1 cm. The electrodes were enclosed in a small chamber (volume ≈ 12.5 mL) to be able to vary a gas composition (gas flow rate ≈ 2 L/min). Transient spark discharge (TS) was driven by positive DC power supply (*Technix RS20*) and its electrical characteristic were monitored by high voltage probe (*Tektronix P6015A*) and Rogowski type current probe (*Pearson Electronics*) connected to an oscilloscope (*Tektronix TDS 1012*). 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.

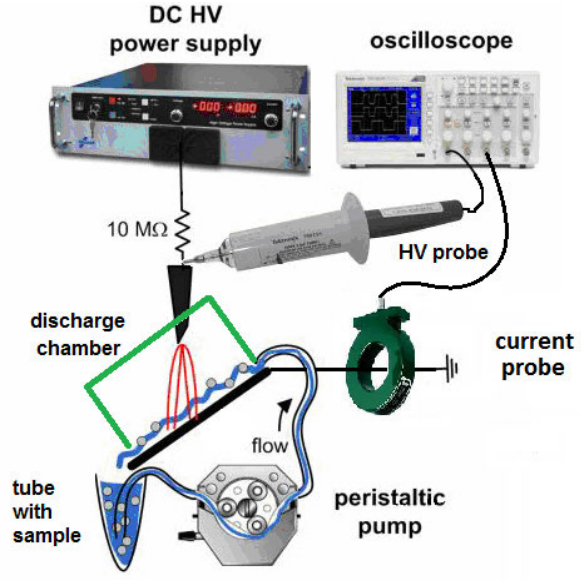

Figure 1. Scheme of experimental set up with water electrode.

Materials and Methods

We investigated chemical effects of the TS discharge in water solutions and biological effects on gram-negative bacteria *Escherichia coli* (CCM3954). The bacteria is well known and widely used as a model microorganism. It is commonly present in intestine of warm-blooded organism, and therefore considered as an indicator of fecal contamination of water. We used planctonic bacterial suspension, i.e. bacteria floating in the solution. The volume of treated samples was 5 mL and the initial concentration of bacteria in the solution was 10^7 CFU/mL (CFU — colony forming unit). The bacterial inactivation was evaluated by standard colony counting method and inactivation effect was expresses as a logarithmic reduction of bacterial concentration.

Chemical effects in water solutions were investigated in non buffered water solution of monosodium phosphate NaH_2PO_4 mimicking tap water (**"water"**, 600 µS/cm, pH 5), as well as in phosphate buffered 2mM solution Na_2HPO_4/KH_2PO_4 (**PB**, 550 µS/cm, pH 7). The concentrations of hydrogen peroxide H_2O_2 and nitrite NO_2^- were evaluated using colorimetric methods. Immediately after the treatment we stabilized the 200 µL of the treated sample by 30 µL of sodium azide NaN_3 to prevent the reaction of H_2O_2 with NO_2^-, and subsequently added 100 µL of titanium oxysulfate $TiOSO_4$. The H_2O_2 reacts with titanyl ions of $TiOSO_4$ and produce yellow-colored product with maximum absorbance peak at 407 nm. To determine nitrite NO_2^- concentration, we employed commercial kit (*Cayman Chemicals*) that uses Griess reagents to form pink-coloured azo-product with maximum absorbance peak at 540 nm. We also monitored the pH (*WTW 3110*), conductivity (*Greisinger Electronic GMH 3430*) and temperature of treated solution approximately 1–2 minutes after the treatment; taking into account the temperature of solution (the post treatment stabilization time was similar for all samples).

Results and Discussion

In this section we present the results of TS discharge biocidal effect on *E. coli* and chemical effects on water solutions ("water", PB), In Figure 2 characteristic voltage and current waveforms of positive DC transient spark discharge are depicted and photograph of the discharge is presented, too. As the figure shows, the TS discharge is typical with current pulses of high amplitude (order of several tens of A) and very short duration (10–100 ns). The frequency of the pulses is usually of order of several kHz that depends on the breakdown voltage. During the current pulses the breakdown voltage drops almost to zero as the electric circuit internal capacity discharges completely. After the breakdown the potential across the discharge gap starts to increase as the capacity recharges until

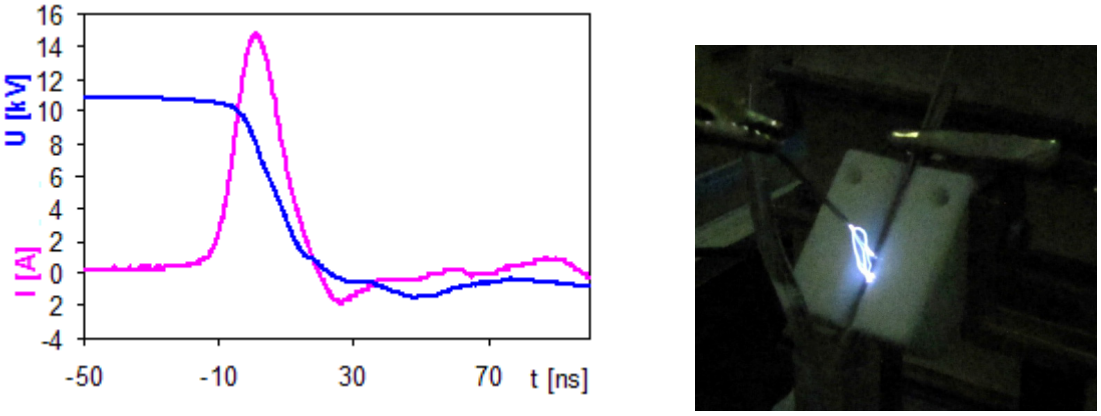

Figure 2. The voltage and current characteristic of DC transient discharge in air at atmospheric pressure, and corresponding photograph of the discharge in water electrode system [ambient air].

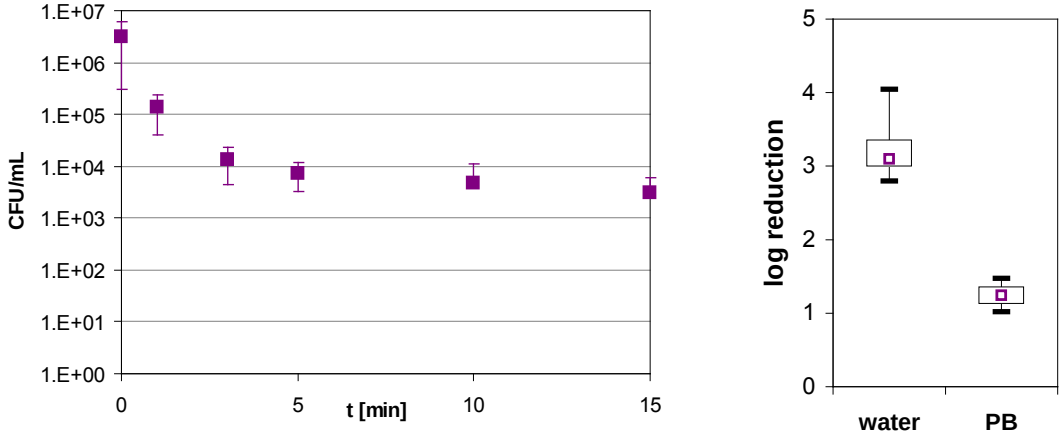

Figure 3. Effect of plasma treatment time on *E. coli* in "water" (left) and comparison of plasma treatment in non-buffered ("water") and buffered (PB) solutions (right) [ambient air, treatment time 5 min, liquid flow 14 mL/min, U_{max} = 11 kV, I_{max} = 10 A, f ≈ 2.5–4 kHz] (data points represents mean value ± standard deviation (left); box and whiskers plot: maximum, upper quartile, median, lower quartile, minimum (right)).

another breakdown occurs. The TS is a self-pulsing repetitive streamer to spark transition discharge [*Janda* 2012], where the repetitive frequency can be well controlled by breakdown voltage.

Figure 3 shows the biocidal effect of TS discharge. A decrease in bacterial concentration from initial 10^7 CFU/mL to 10^4 CFU/mL was observed after 10 minutes treatment of *E. coli* in "water". We achieved stronger inactivation effect in non-buffered solution ≈ 3 log than in buffered ≈ 1 log. To understand different biodical effects of the TS discharge in the two solutions we decided to measure the chemical agents in water potentially responsible for this effect.

In general, plasma can effect its environment by several possible ways — UV radiation, heat, electric field, charged and neutral reactive species. According to several studies [*Herrman* 1999; *Choi* 2006; *Laroussi* 2005] the UV radiation produced by low temperature discharge is not sufficient to inactivate microorganisms at high rates. The bacteria can be efficiently killed by a heat, however in low temperature plasma the gas temperature (represented by the energy of ions) remains almost at ambient temperature. Although the TS discharge produce more heat than other low temperature plasma discharges, in our system after 10 min treatment we observed solution temperature increase only by approximately 4.05 ± 1.5 °C, i.e., very little to have any significant effect on bacteria. Electric field can also affect the cell membrane cause its electroporation and support efficient transport of reactive species into the cell. This phenomenon is more likely dominant in gram-negative bacteria than in gram-positive as the gram-negative bacteria have thinner peptidoglycane layer than gram-

positive. In low temperature plasma the dominant role is often assigned to various atomic and molecular reactive species. Reactive species originates in a gas and dissolve into water or can be generated directly in water. These species change the properties of treated solution. Figure 4 shows dependence of pH and conductivity on plasma treatment time of "water". The decrease of pH is associated with the increase of conductivity. After 15 min treatment pH decreased to 3 and conductivity increased to 1400 µS/cm.

Reactive species responsible for the pH decrease are NO_2^- and NO_3^- (1) that forms when NO_2 generated by the discharge in the gas phase dissolves into water. In the water at acidic pH the NO_2^- can further react and forms NO_3^- dominantly (2). This reaction is however impossible in buffered solution where pH is kept neutral. In this case, NO_2^- is accumulating and therefore its concentration is higher in PB than in "water". In the case H_2O_2 is present; NO_2^- can react with H_2O_2 and form peroxinitrite $ONOO^-$ (3).

$$NO_2(gas) + NO_2(gas) + H_2O \rightarrow NO_2^-(liq.) + NO_3^-(liq) + 2H^+ \quad (1)$$

$$3NO_2^- + 4H^+ + H_2O \rightarrow 2NO + NO_3^- + 2H_3O^+ \quad (2)$$

$$NO_2^- + H_2O_2 \rightarrow O{=}NOO^- + H_2O \rightarrow NO_3^- + H_2O \quad (3)$$

The formation of active species in water is also affected by the gas composition above the water solution. Figure 5 shows the effect of various mixtures of N_2 and O_2. If the discharge is generated in pure oxygen atmosphere with minimum nitrogen impurities, NO_2^- will not formed, its concentration is negligible, and pH will stay constant. On the other hand the concentration of H_2O_2 formed in very high rate in pure oxygen. It is caused by higher formation of hydroxyl radicals that form H_2O_2 and accumulate in water, and also due to lack of NO_2^- they could potentially react with.

In nitrogen atmosphere the discharge produces only a small amount of NO_2^- because of lack of oxygen in reaction, therefore also only a small decrease of pH was observed. In case of O_2/N_2 mixtures in "water" solution, there is most significant pH decrease and the concentration of reactive species is relatively high and balanced (0.4–0.5 mM) compared to pure N_2 and O_2. Synergetic effect of H_2O_2 and NO_2^- in acidic conditions can lead to formation of peroxinitrites and is responsible for overall strong bactericidal effect. On the other hand in PB the pH does not change much (Fig. 5), the concentrations of H_2O_2 is small compared to "water", while concentration of NO_2^- is higher due to neutral pH that suppress nitrites to be converted into nitrates (Fig. 6).

All previous experiments were performed with constant solution flow rate of 14 mL/min. At this flow rate we got almost pulsation-free solution flow and highly repeatable discharge pulses with almost regular frequency. Nevertheless we also investigated the possible effect of solution flow rate on the formation of reactive species. As can be seen in Figure 7, the solution flow rate in the range of

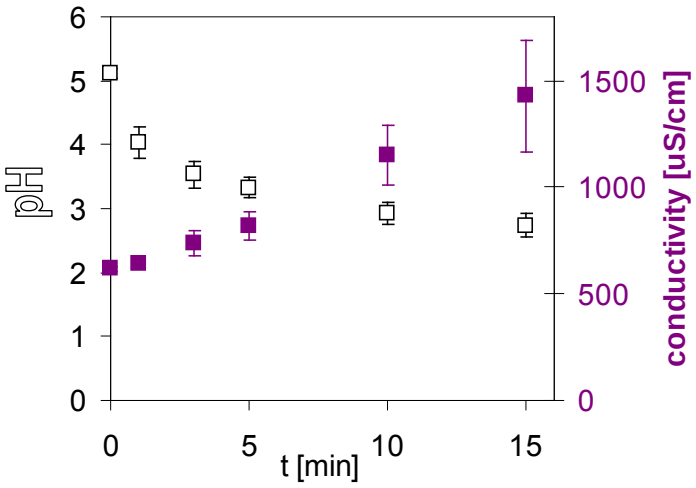

Figure 4. Effect of plasma treatment time on pH and conductivity of 'water' solution [ambient air, liquid flow 14 mL/min, U_{max} = 11 kV, I_{max} = 10 A, f ≈ 2.5–5 kHz] (data points represents mean values ± standard deviation).

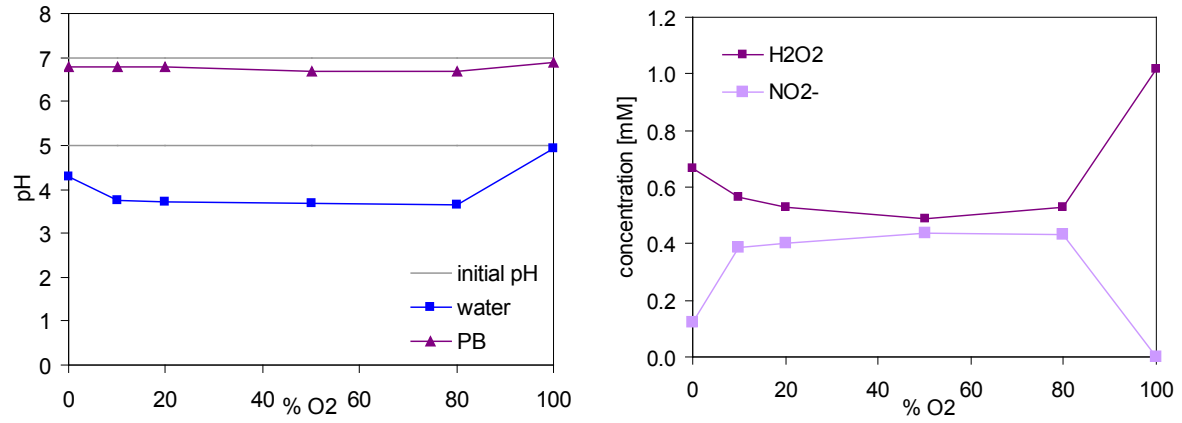

Figure 5. Effect of gas mixture on pH of "water" and PB (left) and concentration of H_2O_2 and NO_2^- in "water" [treatment time 5 min, %O_2 in N_2, U_{max} = 11 kV, I_{max} = 11–18 A, f ≈ 2–4 kHz].

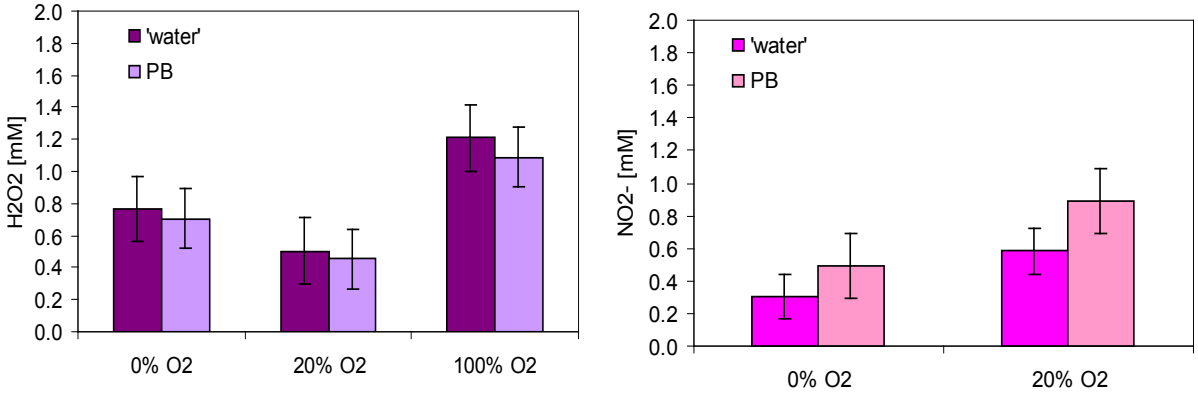

Figure 6. Effect of gas mixture on concentration of H_2O_2 and NO_2^- in "water" and in PB solutions; [treatment time 5 min U_{max} = 11–13 kV, I_{max} = 10–18 A, f ≈ 2.5–4 kHz] (data points represents mean values ± standard deviation).

5–20 mL/min did not have any significant effect on the concentration of the generated species. At very high flow rates the effect on H_2O_2 concentration was observed, however probably the result of irregularity of water flow driven by the pump. The results show the solution flow rate does not have any significant effect and the only parameter that determines the efficiency is the energy density [J/L] delivered to the given volume of the liquid in a given time.

To compare our results with the works of others authors, one has to keep in mind there has been various types of discharges used and utilized in various experimental conditions and therefore comparison of the results is often very difficult or impossible. Many authors uses DBD discharges, particular plasma jets [*Tresp* 2013, *Zhang* 2013, *Akishev* 2015, Xu 2015], which are more gentle in their effect toward water solution containing bacteria, however they demand noble gases as a working gas. Few others utilized a gliding arc [*Burlica* 2006], which induces higher concentration of reactive species in comparison to plasma jets, but these discharges reach higher temperatures, therefore induce different concentration of particular RONS. Treatment of the liquids is often done in a batch mode, rather than continuous mode. There are very few papers where water is circulated [*Jamroz* 2014, *Kovačević* 2014], or even re-circulated as it is in our system.

In many works the achieved bacterial inactivation of *E.coli* is higher [*Pavlovich* 2014, *Oehmigen* 2010, *Joshi* 2011], however the treated volume, the treatment time and used power have to be considered. Pavlovich et al. [*Pavlovich* 2014] used spark-like discharge to produce ≈1 mM NO_2^- concentration in saline solution (0.85 % NaCl, 0.15 mL) after 5 minutes of treatment in air

atmosphere. In NaH_2PO_4 solution we achieved slightly smaller concentration (≈ 0.6 mM), however in larger volume of treated liquid (5 mL). Burlica et al. using the gliding arc discharge [*Burlica* 2006] did not find any hydrogen peroxide in nitrogen or air atmosphere, while in oxygen they produced very less H_2O_2 (0.24 mM) in comparison to our system. On the other hand the concentration of NO_2^- was higher. The pH decrease and NO_2^- chemistry had similar tendency in dependence on gas mixtures. They also observed more obvious pH decrease in air (to pH 2.7) than in oxygen (to pH 3.4). The concentration of NO_2^- was highest in air and they did not detect any NO_2^- in oxygen atmosphere. Jamroz et el. [*Jamroz* 2014] used flowing liquid cathode system and they observed similar concentration of H_2O_2 (1.12 mM) and less concentration of NO_2^- (8.15 μM). Oehmigen et al. [*Oehmigen* 2010] used surface DBD discharge and induced 9 μM of H_2O_2 and 30 μM of NO_2^- in 5 mL of deionized water after 5 min of treatment. Lukeš et al. [*Lukeš* 2014] generated lower concentrations of 120 μM of H_2O_2 and 50 μM of NO_2^-, but in much bigger liquid volume by pulsed discharge generated above a liquid.

In conclusion the water electrode system that we used has a great advantage in its ability to work in ambient air and repetitive contact of liquid with discharge. It allows us to vary liquid volume, liquid flow rate, as well as the treatment time. The reactive species which are produced in air are in good balance and sufficient concentration to inactivate bacteria, considering the volume of bacterial solution, treatment time and power.

Conclusion

We investigated the effects induced in water solutions by positive DC transient spark discharge generated in atmospheric pressure and compare it to other authors work. We achieved 3-log reduction of *Escherichia coli* after 10 min treatment of "water" by TS discharge generated in air. In buffered PB solution we observed lower 1-log reduction. In monosodium phosphate solution decrease of pH from 5 to 2.5, increase of conductivity from 600 to 1400 μS/cm and insignificant increase of temperature were noticed after 15 minutes treatment. Chemical effects, i.e. concentration of H_2O_2 and NO_2^- were measured in dependence on working gas in which was the TS discharge generated. We found the chemical effects are independent on solution flow rate. More experiments are needed to clarify impact of reactive species on bacterial inactivation.

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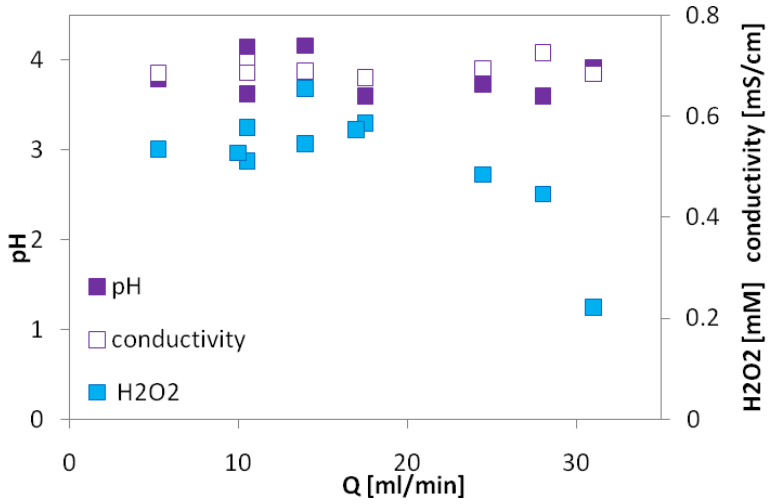

Figure 7. Effect of solution flow rate on conductivity [mS/cm], pH and concentration of H_2O_2 [mM]; [treatment time 5 min, treated volume 5 mL, working gas — air, U_{max} = 10 kV, f ≈ 4 kHz].

References

Akishev, Y., Balakirev, A., Grushin, M., Karalnik, V., Kochetov, I., Napartovich, A., Petryakov, A., Trushkin, N., Long Plasma Jet Generated by DC Discharge in N_2 at Atmospheric Pressure: Impact of Trace Admixtures on Composition of Reactive Species in Far Afterglow, *IEEE Trans. Plasma Sci.* 43, 745–752, 2015.

Burlica, R., Kirkpatrick, M.J., Locke, B.R., Formation of reactive species in gliding arc discharges with liquid water, *J. Electrostat.* 64, 35–43, 2006.

Choi, J.H., Han, I., Baik, H.K., Lee, M.H., Han, D.W., Park, J.C., Lee, I.S., Song, K.M. & Lim, Y.S., Analysis of sterilization effect by pulsed dielectric barrier discharge. *J. Electrostat.* 64, 17–22, 2006.

Fridman, G., Friedman, G., Gutsol, A., Shekhter, A.B., Vasilets, V.N., Fridman, A., Applied plasma medicine. *Plasma Process. Polym.* 5(6), 503–533, 2008.

Hensel, K., Kučerová, K., Tarabová, B., et al., Effects o fair transient spark discharge and helium plasma jet on water, bacteria, cells, and biomolecules, *Biointerphases* 10, in press, 2015.

Herrman, H.W., Henins, I., Park, J.& Selwyn, G.S., Decontamination of chemical and biological warfare, (CBW) agent using an atmospheric pressure plasma jet (APPJ). *Phys. Plasmas*, 6, 2284–2289, 1999.

Jamróz, P., Greda, K., Pohl, P., Zyrnicki, W., Atmospheric Pressure Glow Discharges Generated in Contact with Flowing Liquid Cathode: Production of Active Species and Application in Wasterwaater Purification Processes, *Plasma Chem. Plasma Process.* 34, 25–37, 2014.

Janda, M., Machala, Z., Niklová, A., Martišovitš, V., The streamer-to-spark transition in a transient spark: a dc-driven nanosecond-pulsed discharge in atmospheric air, *Plasma Sources Sci. Technol.* 21 (6), 065002, 2012.

Joshi, S.G.., Cooper, M., Yost, A., Paff, M., Ercan, U.K., Fridman, G.., Friedman, G., Fridman, A., Brooks, A.D., Nonthermal Dielectric-Barrier Discharge Plasma-Induced Inactivation Involves Oxidative DNA Damage and Membrane Lipid Peroxidation in *Escherichia coli*, *Antimicrob. Agents Chemother.* 55, 1053–1062, 2011.

Kovačević, V.V., Dojčinović, B.P., Šupica, D., Jović, M., Sretenović, G..B., Roglić, G..M., Obradović, M., Kuraica, M.M., Formation of Reactive Species in Water Treated by Water Falling Film DBD in Different Gases, *14th International Symposium on High Pressure Low Temperature Plasma Chemistry*, 322–326, 2014.

Laroussi, M., Nonthermal decontamination of biological media by atmospheric-pressure plasmas: Review, analysis, and prospects. *IEEE Trans. Plasma Sci.* 30, 1409–1415, 2002.

Laroussi, M., Low temperature plasma-based sterilization: Overview and state-of-the-art. *Plasma Process. Polym.* 2, 391–400, 2005.

Laroussi, M., Low-Temperature Plasmas for Medicine? *IEEE Trans. Plasma Sci.* 37 (6), 0093–3813, 2009.

Lukeš, P., Lock, B.R., Brisset, J-L., Aquaeous-Phase Chemistry of Electrical Discharge Plasma in Water and in Gas-Liquid Environments. In P. L.-L. Lukeš, & M. M. edited by Vasile I. Parvalescu (Ed.), Plasma Chemistry and Catalysis in Gases and Liquids (1st edition. vyd., kap.7). Wiley-VCH Verlag GmbH & Co. KGaA, 2012.

Lukeš, P., Dolezalova, E., Sisrova, I., Clupek, M., Aqueous-phase chemostry and bactericidal effects from an air discharge plasma in contact with water: evidence for the formation of peroxynitrite through a pseudo-second-order post-discharge reaction of H_2O_2 and HNO_2, *Plasma Sources Sci. Technol.* 23, 15, 2014.

Machala, Z., Tarabová, B., Hensel, K., Špetlíková, E., Šikurová, L., Formation of ROS and RNS in Water Electro-Sprayed through Transient Spark Discharge in Air and their Bactericidal Effects. *Plasma Process. Polym.* 10 (7), 649–659, 2013.

Morent, R., De Geyter, N., Inactivation of Bacteria by Non-Thermal Plasmas. Biomedical Engineering — Frontiers and Challenges. Ed. Prof. Reza Fazel, ISBN: 978-953-307-309-5, 2011.

Oehmigen, K., Hähnel, M., Brandenburg, R., Wilke, Ch., Weltmann, K.-D., von Woedtke, Th., The Role of Acidification for Antimicrobial Activity of Atmospheric Pressure Plasma in Liquids, *Plasma Process. Polym.* 7, 250–257, 2010.

Park, G.Y., Park, S.J., Choi, M.Y., Koo, I.G., Byun, J.H., Hong, J.W., Sim, J.Y., Collins, G.J., Lee, J.K., Atmospheric-pressure plasma sources for biomedical applications. *Plasma Sources Sci. Technol.* 21, 21, 2012.

Pavlovich, M.J., Ono, T., Galleher, C., Clark, D.S., Machala, Z., Graves, D.B., Air spark-like plasma source for antimicrobial NO_x generation, *J. Phys. D: Appl.Phys.*, 47, 10, 2014.

Tresp, H., Hammer, M.U., Weltmann, K.-D., Reuter, S., Effect of Atmosphere Composition and Liquid Type on Plasma-Generated Reactive Species in Biologically Relevant Solutions, *Plasma Medicine*, 3, 45–55, 2013.

Zhang, Q., Liang, Y., Feng, H., Ma, R., Tian, Y., Zhang, J., Fang, J., A study of oxidative stress induced by non-thermal plasma-activated water for bacterial damage, *Appl. Phys. Lett.* 102, 5, 2013.

Xu, D., Liu, D., Wang, B., Chen, Ch., Chen, Z., Li, D., Yang, Y., Chen, H., Kong, M.G.., In Situ OH Generation from O_2^- and H_2O_2 Plays a Critical Role in Plasma-Induced Cell Death, *Plos One*, 10, 14, 2015.