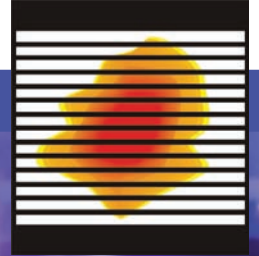


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CHEMICAL AND BACTERICIDAL EFFECTS INDUCED IN WATER TREATED BY AIR TRANSIENT SPARK

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Chemical changes in water induced by DC transient spark discharge generated in atmospheric pressure air and related bactericidal effects were investigated. Changes of pH, conductivity and formation of reactive oxygen (ROS) and nitrogen (RNS) species were observed in aqueous solution contaminated with *E. coli* after treatment by water electro-spray system or water electrode system. It seems that the chemical effects were enhanced in water electrode system and the stronger bactericidal efficiency was observed in the water spray system due to the electro-spray. We found out that the indigo method for measuring ozone in water is not selective in plasma treated solutions.

1 Introduction

Bio-decontamination by cold plasma under wet condition is very important. 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 such as hydrogen peroxide H_2O_2 , hydroxyl radical $\cdot OH$, nitrites NO_2^- , nitrates NO_3^- , hypochlorite OCl^- and peroxyntirites $ONOO^-$ induce chemical changes in water solutions and various biocidal effects on microorganisms or therapeutic effects for biomedical applications [1,2]. It was shown that the acid environment with plasma agents (especially hydrogen peroxide, nitrites, nitrates) lead to the strong bacterial inactivation [3]. Also according to many papers, key species responsible for bio-decontamination in plasma treated water solutions or tissue and cell injuries *in vivo* may be peroxyntirites [4,5]. However, it is not clear enough yet, which ROS/RNS play dominant role in bio-decontamination and biomedical applications of cold plasma.

In this work we investigated chemical effects induced by plasma gas-liquid chemistry in treated aqueous solutions. We measured formation of hydrogen peroxide, nitrites, nitrates, “ozone” and peroxyntirites in water treated by DC-driven positive transient spark discharge. Production of reactive oxygen and nitrogen species was correlated with the changes of pH and electrolytic conductivity. Bactericidal efficiency was observed on *E. coli* suspended in plasma treated solutions. These chemical and bactericidal effects were investigated in two different systems: electro-spray water system [6] and water electrode system. The chemistry was also investigated in synthetic prepared aqueous solution that should simulate the plasma treated water.

2 Experimental set-up and methods

The experimental set-up of water spray and water electrode system is depicted in Figure 1. The main difference between these systems was that in the water spray (WS) system, the water was electro-sprayed through the active zone of the discharge and in the water electrode (WE) system, the water was repetitively circulated by peristaltic pump. DC transient spark discharge was generated in ambient air in point-to-plane geometry. Hypodermic hollow needle represented the high voltage (HV) electrode, which in the WE system is perpendicular to the water level and in the WS system enabled flowing the contaminated water through the discharge zone. In the WS system the treated water passed the inter-electrode gap once and then was collected under the metallic mesh in contrary to the WE system, where the water circulated for 28 times during the treatment. In both systems we tried to reach the similar conditions: voltage (10-13 kV), spark pulse frequency (~ 1 kHz), inter-electrode gap (10 mm), time of the treatment (10 min) and treated volume of water (5 mL). The water flow rate was different: 0,5 mL/min in the WS system and 14 mL/min in the WE system. A positive DC HV was applied through the ballast resistor R (10 M Ω). The voltage signal was measured by the HV probe *Tektronix P6015A* and the current signal was measured on 1 Ω resistor or by Rogowski current monitor *Pearson 2877*. These signals were processed by a digitizing 200 MHz oscilloscope *Tektronix TDS 2024*.

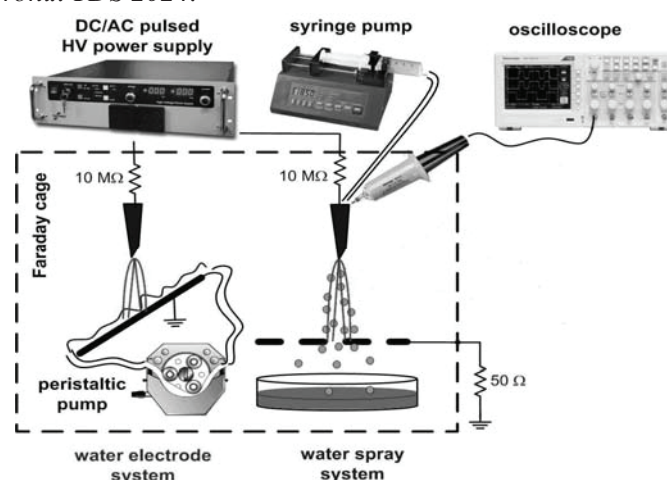


Figure 1: The experimental set-up of water electrode and water spray systems.

Aqueous solutions with different initial pH and electrolytic conductivities σ were used for plasma treatment. The solutions were prepared by the dissolution of different salts in deionized water:

- **“water”**= NaH₂PO₄ solution (pH 5 and $\sigma = 0.6$ mS/cm) mimics pH and conductivity of tap water and has similar chemical composition with the phosphate buffer but no buffering capacity,
- **“PB”**= Na₂HPO₄/KH₂PO₄ buffer (pH 6.8 and $\sigma = 0.56$ mS/cm),
- **solution of 0.7 mM H₂O₂ + 0.7 mM NaNO₂** (at pH 3.3 (in H₃PO₄) or 6.8 (in PB)) = simulates the chemistry of the plasma treated water without plasma.

The measurements of ROS and RNS in plasma treated water were performed by colorimetric methods. UV/VIS absorption spectrometer *UV-1700 SHIMADZU* and fluorescence spectrometer *Perkin Elmer LS 45* were used.

- Titanyl ions Ti^{4+} react with H_2O_2 in the presence of NaN_3 and create perititanic acid with the absorption maximum at 407 nm.
- NO_2^- react with Griess reagents and create azo-dye with the absorption maximum at 540 nm. NO_3^- were converted to NO_2^- utilizing nitrate reductase enzyme.
- Indigo dye with the absorption maximum at 600 nm reacts with O_3 and the colorless product isatin is created by the bleaching process.
- 2,7-dichlorodihydrofluorescein diacetate (prepared according to [7]) after oxidation by peroxyntrites creates the highly fluorescent dichlofluorescein (DCF) with the excitation maximum at 502 nm and emission maximum at 521 nm. The selectivity of this method is still under our investigations [6].

Changes of pH and electrolytic conductivity were measured by pH and conductivity probes (*Adwa, Greisinger Electronic*).

Biocidal effects were tested on Gram negative *Escherichia coli* (CCM 3954) as overnight cultivated bacteria or pre-cultivated on gel discs. Aqueous solutions contaminated with planktonic form had the initial population 10^6 - 10^8 colony forming units per milliliter (CFU/mL). The number of bacteria cells after treatment was assayed by colony forming units cultivated on agar plates.

3 Results and discussion

3.1 Transient spark discharge (TS)

Transient spark discharge was operated in atmospheric air in electro-spray or water electrode systems in contact with water. Electric parameters and emission spectra of TS were documented in detail in our previous works [8,9]. In the WS system, water was sprayed through the HV hollow needle with the water flow rate 0.5 mL/min and in the WE systems water was circulated with the flow rate 14 mL/min, which enables increase the number of the discharge contacts with the treated water.

3.2 Chemistry of plasma treated water linked with the bactericidal efficiency

Escherichia coli suspended in non-buffered and buffered water solutions with different initial pH and electrolytic conductivity was treated by TS discharge. The bactericidal efficiency was expressed as a logarithmic reduction (Fig. 2). In both systems the stronger bactericidal effect was observed in non-buffered solution (3-5 log) than in phosphate buffer solutions (1-2 log). In the WS system, the efficiency was higher than in the WE system. This was probably due to the effect of the electro-spray, where the treated water is sprayed to micrometric size droplets which enhance the mass transfer of ROS/RNS formed in the gas phase into water. The higher efficiency observed in non-buffered solutions was linked with the different ROS/RNS chemistry associated with acidification (pH ~ 3) and increase of the conductivity (0.6→0.8 mS/cm; 6→6.5 mS/cm) observed in both systems. In PB and PBS solutions the bactericidal efficiency was lower with almost no decrease of pH and very low increase of conductivity, which showed the important role of pH in plasma induced bactericidal effects [6].

Because the bactericidal effects of plasma treated water and the decrease of pH are accompanied with the chemical changes, we focused on the formation of ROS and RNS: H_2O , NO_2^- , NO_3^- , O_3 , $ONOO^-$. In both systems were measured H_2O_2 and $ONOO^-$ in non-buffered solutions after plasma treatment. The results showed higher concentrations of $ONOO^-$ and slightly higher concentrations of H_2O_2 in the WE system than in WS system.

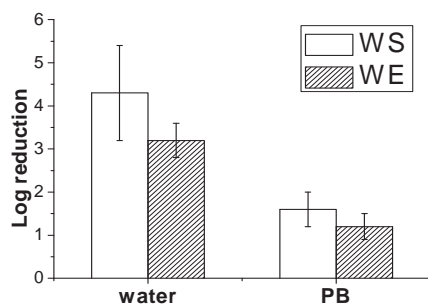


Figure 2: Bactericidal effects induced in the WE and the WS systems.

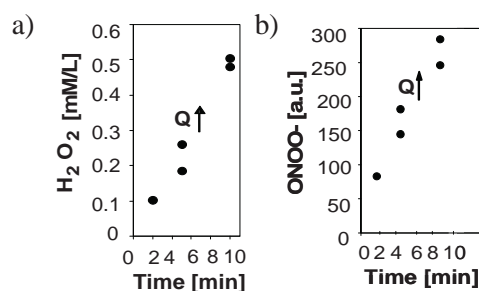
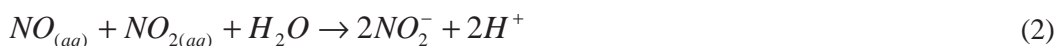
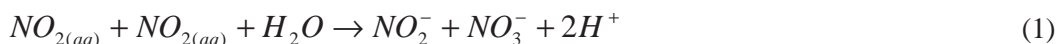


Figure 3: Increase of formation of H_2O_2 and $ONOO^-$ in water in the WE system.

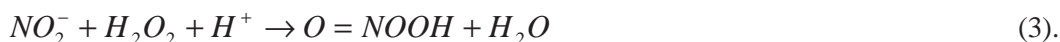
Figure 3 shows that in the WE system, the concentration of H_2O_2 (a) and $ONOO^-$ (b) increased with the increasing time of plasma treatment. It seems that the chemical changes are enhanced in the WE system due to the repetitive circulation of the treated solutions. Formation of nitrites and nitrates was measured by Griess reagents in WS system. Dissolution of NO_x along with the formation of NO_2^- , NO_3^- is responsible for the acidification of the plasma treated solution:



Nitrites produce radicals NO^\cdot and NO_2^\cdot via their protonated form HNO_2 or peroxyntirites via reaction with H_2O_2 , which are also very reactive through their decay products. Therefore, so called *acidified nitrites* under acidic conditions are responsible for the bactericidal properties [1,5,10].

3.3 Chemistry in the simulated plasma treated water

The water solution of 0.7 mM H_2O_2 + 0.7 mM $NaNO_2$ (at pH 3.3 or 6.8) is suppose to simulate the plasma treated water (or PB) [5]. We measured the time developments of H_2O_2 , NO_2^- and ozone, as shown in Figure 4. At pH 6.8 (Fig. 4b), the concentrations were time-stable, unlike at pH 3.3 (Fig. 4a), where we observed the decrease of all three observed reactive species. The explanation of the decrease of the concentrations of H_2O_2 and NO_2^- in time is the reaction of the formation of peroxyntirites in plasma treated solutions [5]:



We observed the dissolved ozone in the plasma treated water by indigo method. We detected 0.6-0.8 mg/L of ozone in plasma treated deionized water by the WS system. Because TS discharge treatment bleached the indigo more than pure ozone bubbled through the deionized water in much higher concentrations, we had to check the

selectivity of the indigo method in the simulated plasma treated water solution with similar chemical composition and pH as our plasma treated water but with no presence of ozone. Interestingly, we detected “ozone” even in this simulated solution without plasma treatment. The degradation of the indigo dye in solution without ozone could be due to the formation of hydroxyl radical $\cdot OH$ created as a decay product of peroxyxynitrites [5, 12]:

$$O = NOOH \leftrightarrow \cdot OH + NO_2 \quad (4).$$

So, the indigo method is not selective to ozone in the plasma treated water.

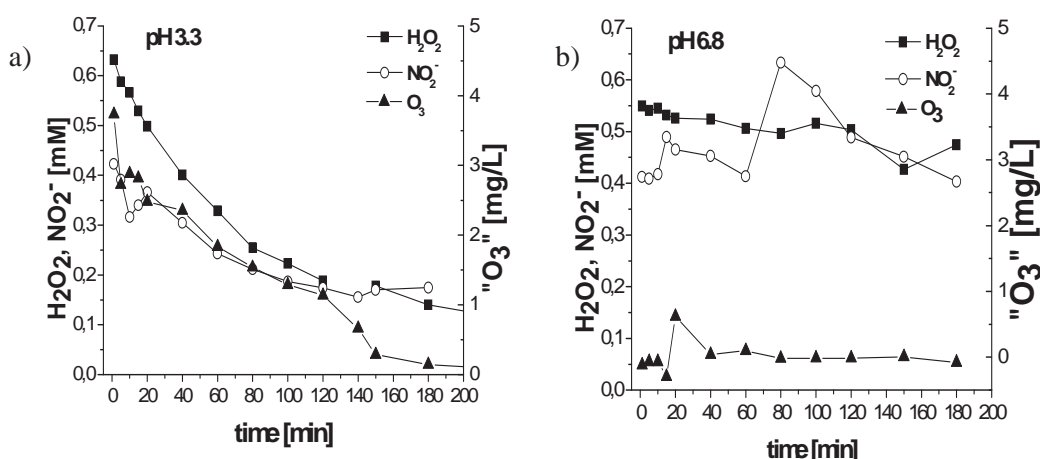


Figure 4: Time developments of important reactive species in the simulated plasma treated water at pH 3.3 (a) and pH 6.8 (b).

Acknowledgements

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