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PLASMA TREATED WATER AS A TOOL FOR SUSTAINABLE APPLICATIONS

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Abstract. Plasma treated water was prepared by non-thermal plasma systems using plasma interaction above or inside liquid or in a remote bubbling regime. Plasma treated water prepared from distilled, tap or water solutions was characterised by physical properties (pH, conductivity) and colorimetric determination of stable chemical species (hydrogen peroxide, nitrites, and nitrates). Its quality was evaluated with respect to its possible utilization in sustainable agriculture and medicine applications.

Keywords: non-thermal plasma, plasma-liquid interactions, oxygen and nitrogen reactive species, colorimetry, plants, *Escherichia coli*.

1. Introduction

Interactions of plasma with liquids induce both physical changes as well as formation of various chemical species containing oxygen, nitrogen or hydrogen that have high redox potential [1]. These species are called RONS (reactive oxygen and nitrogen species), and they can be divided into the short-lived species (such as oxygen, hydrogen or hydroxyl radicals, etc.) and into the long-lived species with considerably longer lifetime in water (hydrogen peroxide, nitrites, nitrates, peroxynitrites, etc.) that might be utilised in subsequent applications [2, 3]. However, composition of such plasma treated water (PTW) and its stability varies according to the way of plasma-liquid interactions as well as according to the quality of the original water (composition, conductivity, pH, etc.) [4–7]. Higher concentration of hydrogen peroxide, nitrites or peroxynitrites in plasma treated water provides antimicrobial properties [2] whereas in lower concentration, stimulation of cell growth was observed [8]. Therefore, plasma treated water or solution rich in these species can be applied in medical applications as a sterilisation medium and wound healing support. On the other hand, higher concentration of nitrogen species such as nitrates stimulate plant growth and together with hydrogen peroxide, it increases germination by changes of the seed shell structure and wettability. Then, such plasma treated water can be used in agricultural applications [9, 10]. The advantage of such prepared medium is that it does not contain additional chemicals burdening the environment, and it is fully biodegradable.

This paper compares quality of plasma treated water prepared in four different plasma devices using non-thermal plasma interaction above or inside the treated liquid or in the remote bubbling regime. PTW was prepared from distilled or tap water or water solution (physiological or nutrient), according to its intended use for agricultural or medical applications. Preliminary results confirming stimulation effects of PTW on the plant growth of lettuce (*Lactuca sativa*) and onion (*Allium cepa*) are presented, both for the treated tap water and treated nutrient solution. Antimicrobial effects of PTW prepared from the distilled water in the DBD plasma system were proved on the gram-negative bacteria *Escherichia coli*.

2. Experimental

2.1. Plasma systems for plasma treated water preparation

Four plasma systems generating plasma either above water surface (microwave plasma jet and dielectric barrier discharge with the liquid electrode) or inside liquid (pin-hole discharge) or as the remote plasma device with bubbling of its gaseous products into the liquid were used for the plasma treated water preparation.

The microwave plasma jet (MW) operating at low power of 8–15 W (frequency of 2.45 GHz) was already described in [11]. The plasma flow in the quartz capillary (the inner/outer diameter of 3/8 mm) produced a surface wave discharge in argon (purity of 99.99 %; gas flow of 2–5 l min^{-1}). This plasma device has been commonly used for direct non-thermal plasma treatments of thermally sensitive materials (even living tissues). Preparation of plasma treated water was carried out by the plasma-liquid interaction above the liquid surface of distilled water or physiological solution (treated volume of 50 ml; treatment time of 5 min).

Dielectric barrier discharge with the liquid electrode (DBD) also produced the non-thermal plasma above the liquid surface of the treated water. However, no additional gas flow was supplied into the reactor and plasma was created in the ambient air. This device was specially constructed for the PTW preparation in agricultural applications, and it was already described in [12]. It was operating at high frequency voltage (frequency of 11 kHz; peak-to-peak voltage of 16 kV) with the total power supply of (36±2) W. Distilled water or physiological solution was used (volume of 75 ml; treatment time of 2, 5 or 10 min).

The plasma device generating the non-thermal plasma directly in liquids was based on the combined configuration of the pin-hole and corona discharge. Its construction was patented by both Czech and European patent [13, 14], and it was described in [15]. It was operating with the DC high voltage source (positive non-pulsing voltage up to 1 kV on the main electrode; power of 50 W; DC+) or with the AC high frequency voltage source (frequency of 15 kHz; peak-to-peak voltage of 16 kV; power of 40 W; AC). The plasma treated water was prepared from distilled water (in the AC discharge, only), tap, physiological or nutrient solution (treated volume of 50–200 ml; treatment time of 1 or 2 min).

The last plasma system used a commercial device ozoniser Lifepool 0.5 (O_3) based on the dielectric barrier discharge (Lifetech s.r.o.; supplied power of 32 W). The gas flow of the artificial dry air (oxygen and nitrogen in the ratio 1:4) was set by mass flow controllers to 0.1 l min^{-1} (oxygen) and 0.4 l min^{-1} (nitrogen), respectively. The discharge gaseous products were let bubbled into the distilled or tap water (treated volume of 150 ml; treatment time of 2, 5 or 10 min). Plasma treated water prepared in this system contained considerably higher amount of nitrogen species comparing to other devices and together with the possibility to enhance the treated volume almost unlimitedly, it represented an interesting alternative of nutrient source utilized in agricultural applications.

2.2. Water solutions

As a liquid precursor, four kinds of water or water solutions were used with respect to its application in medicine or agriculture: distilled (DW) and tap water (TW), physiological solution (PS), and nutrient solution (NS). Initial pH / conductivity of these liquids was following: DW 6.5 / 1.5 µS cm^{-1}, TW 7.1 / 450 µS cm^{-1}, PS 6.0 / 14 900 µS cm^{-1}, NS 6.9 / 500 µS cm^{-1}.

Physiological solution used for medicinal applications (antimicrobial affects) contained 9 g l^{-1} NaCl in distilled water. Nutrient solution used for ecotoxicological tests (growth of *Lactuca sativa*) was composed according to the regulation ISO 6341: 2.59 g l^{-1} $NaHCO_3$, 11.76 g l^{-1} $CaCl_2 \cdot 2H_2O$, 4.93 g l^{-1} $MgSO_4 \cdot 7H_2O$, and 0.23 g l^{-1} KCl.

2.3. Characterisation of plasma treated water and solutions

Characterisation of plasma treated water was carried out by measurements of chemical-physical properties (pH, and conductivity) and by colorimetric determination of stable chemical species (hydrogen peroxide, nitrites, and nitrates). For hydrogen peroxide estimation, titanium(IV) oxysulfate in sulfuric acid solution ($TiOSO_4 \cdot (H_2SO_4)_x$; Millipore) was used as a selective reagent, and its concentration was evaluated according to the absorbance of yellow peroxytitanium acid complex at 407 nm [16]. To determine nitrites and nitrates, commercial colorimetric kits for freshwater (Supelco or Tetra, alternatively) were used as selective reagents together with absorption measurements at appropriate wavelengths of 540 nm (nitrites) or 526 nm (nitrates), respectively [17].

2.4. Evaluation of ecotoxicological tests on plants

Effects of plasma treated water on the plant growth were evaluated as the root length of two selected representatives: lettuce (*Lactuca sativa*), and onion (*Allium cepa*). In both cases, the tap water treated by DC+ plasma directly in water (positive voltage on the main electrode) was tested. In the case of lettuce, the nutrient solution was treated by the same plasma source and applied, too.

The lettuce seeds (30 seeds in 5 repetitions) were placed on the filter paper in the Petri dish watered by 6 ml of the tested liquid (plasma treated or control) and let germinated under the cover in dark at temperature range of 22–24 °C for 7 days. Then, the length of roots was measured.

The onion bulbs (the mean diameter of (18±2) mm; the mean weight of (3.0±0.5) g) were gently scuffed and immersed in water at 7 °C for 24 hours to revive germ root cells. Then, the onions were placed on the test tubes filled with the tested liquid (plasma treated or control) so that the bottom part of the bulb was in contact with the water surface. The samples were exposed to the natural day light regime by the window for 7 days. Then, the length of the roots was measured.

2.5. Evaluation of antimicrobial effects

Effects of plasma treated water on microorganisms were tested on the gram-negative bacteria *Escherichia coli*. Concentration of its initial suspension was 10^8 CFU ml^{-1}. In this case, PTW was prepared in the DBD plasma system with the liquid electrode from distilled water. Preparation time was 2, 5 or 10 minutes. Subsequently, 1 ml of the fresh bacterial culture was mixed with 9 ml of each PTW sample and let interact for 10 minutes. Then, 100 µl of the bacterial and PTW mixture was transferred onto agar plates (Lysogeny Broth medium; Lennox) and let incubated at 37 °C for 24 h. Antimicrobial effects were evaluated by the calculation of the number of colony forming units on the plates (CFU method).

3. Results

3.1. Quality of plasma treated water and solutions

Plasma treated water prepared in four plasma systems described above was characterised by colorimetric determination of hydrogen peroxide, nitrites, and nitrates as well as by pH and conductivity measurements. Obtained results are compared in Table 1 for PTW prepared from distilled water and physiological solution. Results from the pin-hole system generating plasma inside the liquid are presented for the high frequency discharge (AC) and positive DC discharge (DC+) separately. In the case of the DC discharge, it was not possible to ignite the plasma in the distilled water due to its low conductivity. Therefore, the data for physiological solution are presented, only. Additionally, as the bubbling system with the remote ozoniser (O_3) is primary intended for agricultural applications, distilled and tap water was used in experiments, only.

Comparing the content of reactive species, we can assume that both systems producing plasma above water surface (i.e., microwave plasma jet (MW) and dielectric barrier discharge with the liquid electrode (DBD)) provided significantly higher amount of nitrogen species, especially nitrites. On the other hand, concentration of hydrogen peroxide was substantially lower than in the discharges generated directly inside the liquid (AC or DC+). In the bubbling system with the ozoniser (O_3), concentration of nitrates was sufficiently increased, only.

Concerning the influence of the initial conductivity, both production of hydrogen peroxide and nitrites was decreased in the physiological solution (initial conductivity of $14.9\,\mathrm{mS\,cm^{-1}}$), except of the DBD system. In all cases, initial conductivity was enhanced while pH was decreased to acidic values. The most acidic conditions were observed in the DBD system which correlated with the higher concentration of nitrites.

To summarize the results with respect to the possible application of particular PTW, both the DBD and the pin-hole systems (AC, DC+) were suitable to produce PTW intended for antimicrobial treatments due to the higher concentration of nitrites and hydrogen peroxide. Microwave plasma jet (MW) was not convenient due to the lower concentration of produced species and substantially higher specific energy supplied for their production. The bubbling system with the ozoniser (O_3) producing higher concentration of nitrates in PTW could be utilised for agricultural applications.

Time stability of PTW composition was also influenced by the plasma system used for its preparation. According to its composition discussed above, selected samples of PTW prepared from distilled water in two most desired systems (DBD above the liquid and AC pin-hole discharge in the liquid) were stored in dark at low temperature of 8 °C. A long time evaluation of

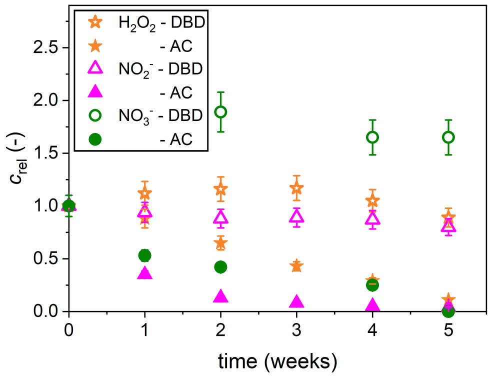

Figure 1. Time stability of plasma treated water prepared from distilled water in the DBD plasma system above the liquid (DBD) and by the AC pin-hole discharge in the liquid (AC). Samples were stored in the dark at low temperature of 8 °C.

relative concentration of RONS is presented in Figure 1. The PTW ageing was dependent on the quality of prepared plasma treated water according to the plasma source. In the case of the DBD system with the relatively lower concentration of hydrogen peroxide, the amount of all determined species remained presented in PTW for weeks. Only, a slight decrease of nitrite concentration with simultaneous increase of nitrates due to the oxidation was observed. On the other hand, concentration of all determined RONS decreased during the storage of PTW from the AC pin-hole system. Due to the higher concentration of hydrogen peroxide, we suppose its reaction with nitrogen species to form peroxonitrite and peroxynitrous acids [18]. Nevertheless, the content of RONS was still significant after the one-week storage (hydrogen peroxide almost 90 %, nitrites 35 %, and nitrates over 50 %). Therefore, we can summarize that PTW prepared in both plasma systems preserved its properties and could be used in the short-term applications (i.e., within a few days).

3.2. Effects of plasma treated water and solutions on plants

Plasma treated water intended for agricultural applications was primary applied via ecotoxicological tests on lettuce seeds and onion bulbs in order to compare influence of PTW on their root length. For this purpose, 200 ml of tap water or nutrient solution was treated by non-thermal plasma of the combined pin-hole and corona discharge generated directly in the liquid by DC high voltage with the positive main electrode (DC+) for 1 min.

Concentration of RONS in plasma treated tap water was as follows: hydrogen peroxide $(12.3\pm1.9)\,\mathrm{mg\,l^{-1}}$, nitrites $(24.2\pm1.2)\,\mathrm{\mu g\,l^{-1}}$, and nitrates $(34.7\pm1.8)\,\mathrm{mg\,l^{-1}}$. Conductivity was increased from $450\,\mathrm{\mu S\,cm^{-1}}$ to $540\,\mathrm{\mu S\,cm^{-1}}$ while pH was decreased from the initial value of 7.1 to 6.6. Con-

Plasma source / Liquid / E$_{spec}$ (kJ l^{-1})	**H_2O_2 (mg l^{-1})**	**NO_2^- (mg l^{-1})**	**NO_3^- (mg l^{-1})**	**G (µS cm^{-1})**	**pH**
MW / DW / 90	under LOD	0.140	1.7	23	5.3
MW / PS / 90	under LOD	0.080	3.3	15 300	5.7
DBD / DW / 58	0.8	1.150	3.5	21	4.5
DBD /PS / 58	0.7	1.340	52.6	15 200	3.6
AC / DW / 32	13.5	1.970	8.2	25	5.4
AC / PS / 48	2.9	0.031	under LOD	16 000	6.1
DC+ / PS / 48	13.6	0.024	0.9	18 000	5.2
O_3 / DW / 26	0.4	0.012	6.2	24	6.4
– / DW / –	0.0	0.0	0.0	1.5	6.5
– /PS / –	0.0	0.0	0.0	14 900	6.0

Table 1. Composition of plasma treated liquids prepared in different plasma sources (MW = microwave plasma jet); DBD = dielectric barrier discharge with the liquid electrode; AC = AC pin-hole discharge in liquid; DC+ = positive DC pin-hole discharge in liquid; O_3 = bubbling system with the ozoniser) from distilled water (DW) and physiological solution (PS). (LOD = limit of detection)

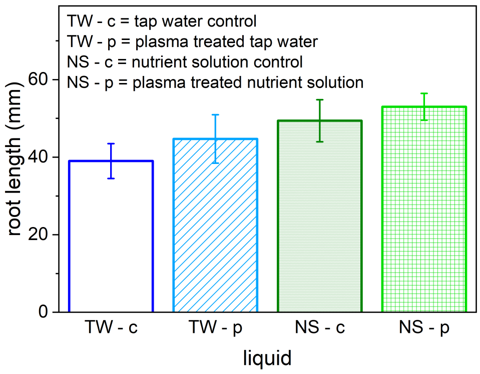

Figure 2. Root length of lettuce (Lactuca sativa) planted in plasma treated water (tap and nutrient solution; DC+ pin-hole system) for 7 days.

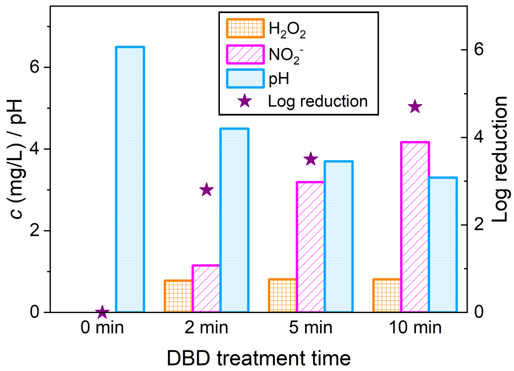

Figure 3. Composition of PTW prepared in the DBD plasma system from distilled water, and its reduction effect on E. coli as a function of the DBD treatment time used for the PTW preparation.

cerning the plasma treated nutrient solution, the content of RONS was following: hydrogen peroxide (4.8±0.6) mg l^{-1}, nitrites (25.3±1.3) µg l^{-1}, and nitrates (8.2±0.4) mg l^{-1}. Conductivity was increased from 500 µS cm^{-1} to 560 µS cm^{-1} while pH was decreased from the initial value of 6.9 to 6.5.

Comparison of the lettuce (*Lactuca sativa*) root length after 7 days in PTW prepared from tap and nutrient solution is demonstrated in Figure 2. In both types of PTW, the root length was prolonged by approximately 10 % with respect to the control sample. Even better results were observed in the case of onion (*Allium cepa*) planted in the tap PTW for 7 days in which the root length was prolonged by 40 %.

3.3. Effects of plasma treated water and solutions on microorganisms

For antimicrobial effects of PTW on the gram-negative bacteria *E. coli*, plasma treated distilled water prepared in the DBD with the liquid electrode was used. Preparation time was set for 2, 5, and 10 minutes which provided different composition of the prepared PTW (Figure 3). While concentration of hydrogen peroxide was low and almost independent on the treatment time, concentration of nitrites was significantly increased with simultaneous decrease of pH during the longer preparation time. As both nitrites and low pH are important antimicrobial factors, we had expected inhibition effects on bacteria. This assumption was proved by the 10-minute interaction of PTW with the bacterial suspension followed by the 24-hour incubation on the agar plates. The decreased number of colony forming units of *E. coli* was clearly visible by the naked eye (Figure 4) as well as by the calculation itself. The results confirmed that the increased preparation time of PTW enhanced bacterial decontamination almost exponentially, i.e. more or less proportionally in the log-scale. The highest 4.7-log reduction was obtained with PTW prepared for 10 minutes in the DBD plasma system (Figure 3).

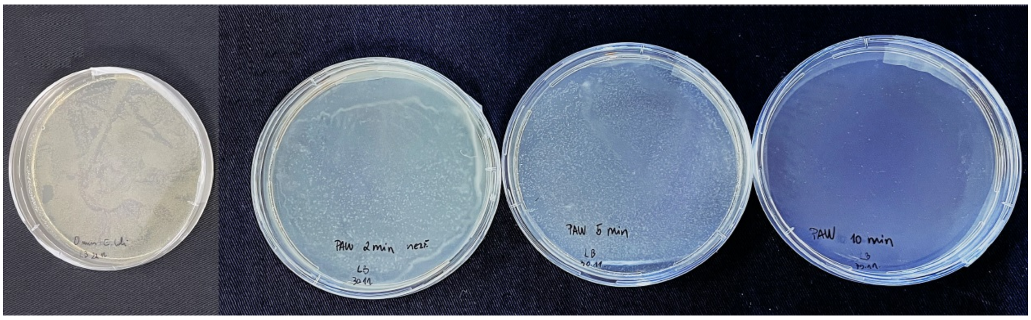

Figure 4. LB agar plates with E. coli after the 10-minute interaction with PTW prepared in the DBD plasma system from distilled water (from the left: DBD treatment time of 0, 2, 5, and 10 min) and 24-h incubation.

4. Conclusions

Quality (given by chemical composition, pH and conductivity) and time stability of plasma treated water was influenced by the plasma system used for its preparation as well as by the original liquid. Higher concentration of nitrogen species was provided by the above liquid systems (the MW plasma jet and the DBD with the liquid electrode) while higher concentration of hydrogen peroxide was produced in the inside liquid systems (with both AC and DC+ voltage). The content of RONS was preserved in PTW for weeks if stored in the dark at low temperature. Higher content of nitrites and hydrogen peroxide together with the decreased pH provided antibacterial effects that were confirmed on the gram-negative bacteria *E. coli*. Positive effects of PTW on plants were confirmed on the root growth of lettuce and onion. Based on the proved effects, PTW with appropriate composition could be utilised as an alternative nutrient or sterilisation medium in sustainable agricultural and medicinal applications.

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References

[1] P. J. Bruggemann et al. Plasma-liquid interactions: A review and roadmap. *Plasma Sources Sci. Technol.*, 25:053002, 2016. doi:10.1088/0963-0252/25/5/053002.

[2] P. Lukeš et al. Aqueous-phase chemistry 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.*, 23:015019, 2014. doi:10.1088/0963-0252/23/1/015019.

[3] J. Julák et al. Contribution to the chemistry of plasma-activated water. *Plasma Physics Reports*, 44(1):125–136, 2018.

[4] K. C. Hsieh et al. Analysis of electrical discharge plasma in a gas-liquid flow reactor using optical emission spectroscopy and the formation of hydrogen peroxide. *Plasma Process. Polym.*, 13:908–917, 2016. doi:10.1002/ppap.201500204.

[5] J.-S. Oh et al. UV–VIS spectroscopy study of plasma-activated water: Dependence of the chemical composition on plasma exposure time and treatment distance. *Jpn. J. Appl. Phys.*, 57:0102B9, 2018. doi:10.7567/JJAP.57.0102B9.

[6] K. Kutasi et al. Tuning the composition of plasma-activated water by a surface-wave microwave discharge and a kHz plasma jet. *Plasma Sources Sci. Technol.*, 28:095010, 2019. doi:10.1088/1361-6595/ab3c2f.

[7] I.-E. Vlad et al. Time stability of water activated by different on-liquid atmospheric pressure plasmas. *Journal of Electrostatics*, 87:284e292, 2017. doi:10.1016/j.elstat.2017.06.002.

[8] C. Duchesne et al. Comparative study between direct and indirect treatment with cold atmospheric plasma on in vitro and in vivo models of wound healing. *Plasma Medicine*, 8(4):379–401, 2018. doi:10.1615/PlasmaMed.2019028659.

[9] F. Judée et al. Plasma-activation of tap water using DBD for agronomy applications: Identification and quantification of long lifetime chemical species and production/consumption mechanisms. *Water Research*, 133:47e59, 2018. doi:10.1016/j.watres.2017.12.035.

[10] P. Škarpa et al. Effect of plasma activated water foliar application on selected growth parameters of maize (Zea mays L.). *Water*, 12:3545, 2020. doi:10.3390/w12123545.

[11] F. Krčma et al. Microwave micro torch generated in argon based mixtures for biomedical applications. *J. Phys. D: Appl. Phys.*, 51:414001, 2018. doi:10.1088/1361-6463/aad82b.

[12] J. Šimečková et al. Influence of plasma-activated water on physical and physical–chemical soil properties. *Water*, 12:2357, 2020. doi:10.3390/w12092357.

[13] F. Krčma. *Jet system for plasma generation in liquids.* Czech Republic Patent CZ305304B6, 2015.

[14] F. Krčma. *Jet system for plasma generation in liquids.* European Patent EP3122161B1, 2019.

[15] F. Krčma et al. Characterization of novel pin-hole based plasma source for generation of discharge in liquids supplied by DC non-pulsing voltage. *Plasma Sources Sci. Technol.*, 27(6):065001, 2018. doi:10.1088/1361-6595/aac521.

[16] G. M. Eisenberg. Colorimetric determination of hydrogen peroxide. *Ind. Eng. Chem., Anal. Ed.*, 15:327–328, 1943.

[17] J. B. Fox. Kinetics and mechanisms of the Griess reaction. *Anal.Chem.*, 51(9):1493–1502, 1979. doi:10.1021/ac50045a032.

[18] I. M. Piskarev et al. Peroxynitrous acid formation induced by air spark plasma radiation. *High Energy Chemistry*, 48(5):350–352, 2014. doi:10.1134/S0018143914050129.