Cold atmospheric pressure plasma generated in contact with a flowing liquid cathode - a new frontier in continuous synthesis of plasma-activated liquid

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Abstract: We show the use of a cold atmospheric pressure plasma (CAPP)-based reaction-discharge system for continuous synthesis of plasma-activated liquid (PAL). Generation of reactive species during CAPP operation was confirmed by optical emission spectrometry (OES). Physicochemical properties of PAL were established. It showed antibacterial properties towards phytopathogenic bacterium *Pectobacterium parmentieri* that is responsible for significant economic losses in potato production worldwide.

Keywords: direct current atmospheric pressure glow discharge, phytopathogen, *Pectobacterium parmentieri*, agriculture

1. Introduction

Plasma is the fourth state of matter together with solids, liquids, and gases. Production of reactive oxygen and nitrogen species (RONS), such as hydrogen peroxide (H_2O_2), ammonium nitrate (NH_4^+), nitrogen oxides (NO_x), hydrogen peroxonitrate (O=NOOH), singlet oxygen ($^1O_2$), ozone (O_3), hydroxyl (OH), hydroperoxyl (O_2H), hydrated electrons (e^-_{aq}), UV lights, and ionized particles, during plasma treatment makes it attractive for proposal of novel innovative applications [1].

Within different types of plasma, one of the most favourable types is cold atmospheric pressure plasma (CAPP), named so because of gas temperature (up to 3,000 K) in which this plasma can be operated [2]. The relatively low temperature of plasma discharge gas, in addition to the RONS generated during CAPP treatment, allow CAPP to be utilized in medicine [3] and agriculture [4]. In more details, CAPP might be used for wound healing [5], blood coagulation [6], inhibition of tumour proliferation [7], purification of agricultural waste from hazardous compounds [8], and eradication of phytopathogenic microorganisms by plasma-activated liquids (PALs) [8,9].

There is a high demand for development of fast, effective, inexpensive and versatile methods targeting plant pathogenic bacteria that affect vegetables, crops and ornamentals. One should have in mind that human population is constantly increasing and providing sufficient amount of nutritious food shall become one of the main challenges of the 21th century. Therefore, we decided to produce PAL in order to benefit from its vast pool of RONS along with solvated electrons (e^-_{aq}), accounting for its antimicrobial properties. Furthermore, application of PALs in agriculture would not trigger to release any toxic chemicals to natural environment, what further supports application of PAL to limit the spread of phytopathogenic microorganisms. Unfortunately, most PALs are produced by operating CAPPs in a stationary reaction-discharge systems [10-13]. In this approach, only small volumes of liquids are treated by CAPP, being responsible for low-efficiency of such systems. The above-listed drawback might be overcome by dedication of a CAPP-based continuous-flow reaction-discharge system for production of PALs.

The main goal of this work was to develop and characterise a new dc-APGD-based method for continuous production of PAL, which shall be afterwards used for inactivation of *Pectobacterium parmentieri*, recently established species of pectinolytic plant pathogenic bacteria that is widespread in Europe nowadays and causes blackleg and soft rot symptoms, being diseases of high economic importance.

2. Materials and Methods

As a PAL precursor, a 0.1 % (m/m) solution of magnesium sulphate ($MgSO_4$, Sigma-Aldrich, Germany) was used. In order to produce PAL, a previously described dc-APGD-based reaction-discharge system was used [8]. In this system (Fig. 1.), stable-in-time dc-APGD was operated in open-to-air atmosphere, in the 5.0 mm gap between the surface of a flowing liquid cathode (FLC) and a pin-type tungsten anode (ID= 4.00 mm). A HV generator (Dora Electronics Equipment, Poland) was applied to maintain dc-HV potential (1100-1300 V) between the anode and the cathode. A constant value of the discharge current, *i.e.* 45 mA, was provided by including a ballast resistor in the anode circuit. The PAL precursor solution was introduced to the dc-APGD-based reaction-discharge system at a flow rate of 3.5 mL min^{-1} through a quartz-graphite capillary (OD = 6.00 mm; ID = 4.00 mm). Electrical contact was ensured by applying a platinum (Pt) wire attached to the quart-graphite capillary. To make the dc-APGD reaction-discharge system suitable for production of PAL, a quartz chamber was used for collecting the resultant product.

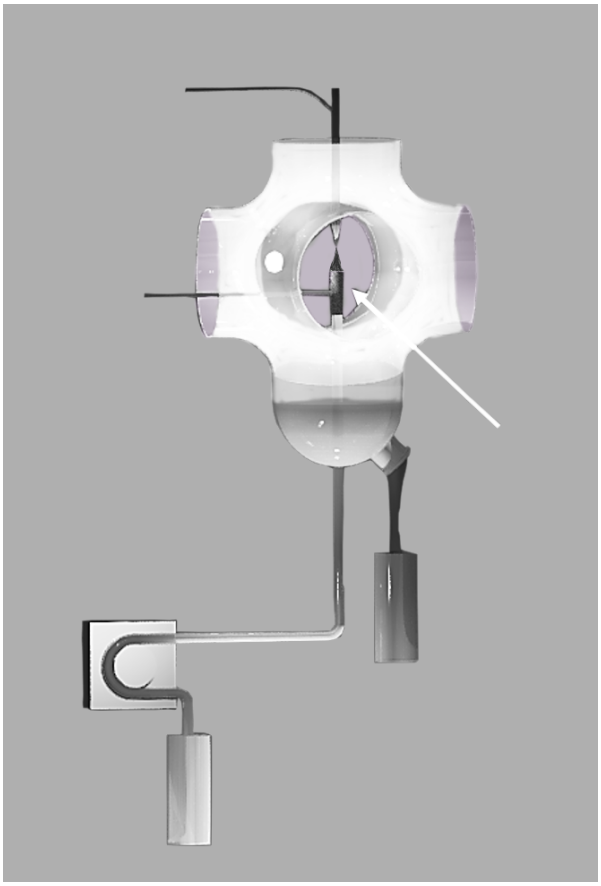

Fig. 1. A schematic representation of dc-APGD-based reaction-discharge system for the continuous synthesis of PAL dedicated for eradication of *P. parmentieri*. The arrow shows dc-APGD, which is a type of CAPP.

Next, reactive species generated during production of PAL in the gas phase of dc-APGD were identified using optical emission spectrometry (OES). The OES spectrum was acquired in the range from 200 to 900 nm by collimating radiation emitted by dc-APGD on the entrance slit (10 μm) of a Shamrock SR-500i (Andor, United Kingdom) spectrometer. A Newton DU-920P-OE CCD camera (Andor, United Kingdom) supported the spectrometer.

To confirm physicochemical properties of PAL, pH and conductivity were measured before and after performing dc-APGD treatment of the $MgSO_4$ solution. A dual CPC-505 pH- and conducto-meter (Elmetron, Poland) was used for that purpose.

To examine suitability of dc-APGD to produce PAL of antimicrobial properties, effects of the post-plasma solution on growth inhibition in the liquid culture of *P. parmentieri* IFB5308 strain was estimated. Briefly, bacteria were grown overnight at 28°C in TSB medium. Then the cells were centrifuged at 6 000 rpm for 10 min, washed twice in sterile physiological saline [0.85% (m/v) NaCl] and optical density of the resultant bacterial solution was adjusted to 0.5 McF. To establish minimal inhibitory concentrations (MICs), bacteria were subjected to diverse concentrations of the obtained PAL, while being suspended in TSB medium within 96-wells microplates. For growth assessment, absorbance at 600 nm was measured by an Envision microplate reader (PerkinElmer, USA), prior and post incubation of the 96-well plates at 28 °C for 24 h. MIC corresponded to the lowest concentration of PAL that efficiently inhibited microbial growth. Subsequently, the pathogenicity assay on potato slices was conducted accordingly to the previously described protocol [14] with minor modifications. In more details, potato tubers were washed in tap water, surface sterilised for 10 min in a 10% commercial bleach and dried under a laminar flow cabinet. Afterwards, these tubers were cut in 1 cm thick slices. Potato slices, on the surface of which 2-3 holes of diameter 0.5 cm were drilled, were placed on moistened filter paper in plastic boxes. Then, 30 μL of the 0.5 McF bacterial solution and 30 μL of PAL was poured to each whole. The boxes were covered with lids and incubated at 28 °C for 48 h prior to measurement of the diameter of rotting spots. Negative and positive controls were included. The experiment involved 9 replicates per sample.

3. Results and Discussion

After dc-APGD treatment of the $MgSO_4$ solution, resultant PAL was gathered in the cathodic compartment of the quartz chamber of the dc-APGD-based reaction-discharge system. Next, OES was used to distinguish individual reactive individuals generated during dc-APGD operation.

Fig. 2. shows the OES spectrum recorded under defined operating conditions of dc-APGD, under which PAL was produced. As can be seen in Fig. 2, numerous bands corresponding to NO (weak $A^2\Sigma^+$-$X^2\Pi$ system), N_2 (strong $C^3\Pi_u$-$B^3\Pi_g$ and weak $B^3\Pi_g$–$A^3\Sigma^+_u$ system), OH (strong $A^2\Sigma$-$X^2\Pi$ system), NH (moderate strong $A^3\Pi$-$X^3\Sigma$ system) and N_2^+ (weak $B^2\Sigma^+$-$X^2\Sigma^+$ system) were noted. The most intense bands were NO (0-1) at 237.0 nm, NO (0-2) at 247.9 nm, OH (0-0) at 308.9 nm, NH (0-0) at 336.0 nm, N_2 (0-0) at 337.1 nm, N_2 (0-1) at 357.7 nm, and N_2^+ (0-0) at 391.4 nm. Atomic lines of H (at 486.2 nm and 656.2 nm) and O (at 777.2 nm and 844.6 nm) were also detected. Additionally, the relatively strong atomic (MgI) line of Mg at 285.21 nm and weak ionic (MgII) lines of Mg at 279.5 nm and 280.2 nm were identified. The emission intensity ionic to atomic line ratio for Mg, *i.e.* MgII/MgI, was calculated to be 0.04. At that condition, the electron temperature (T_e), roughly estimated from the MgII/MgI ratio [15], was 6500 K (0.56 eV). Rotational (gas) temperature (T_{rot}) of the dc-APGD core, calculated from the OH (0-0) spectra for the R_2 branch, was 2900 K (0.25 eV). The estimated electron temperature was almost twice that of the rotational (gas) temperature, namely $T_e > T_{rot}$. This might be caused by a non-equilibrium state of dc-APGD generated in contact with the PAL precursor flowing solution. In summary, the excited states of N_2^+,

H, NO, N_2, OH, and O, were produced during dc-APGD operation.

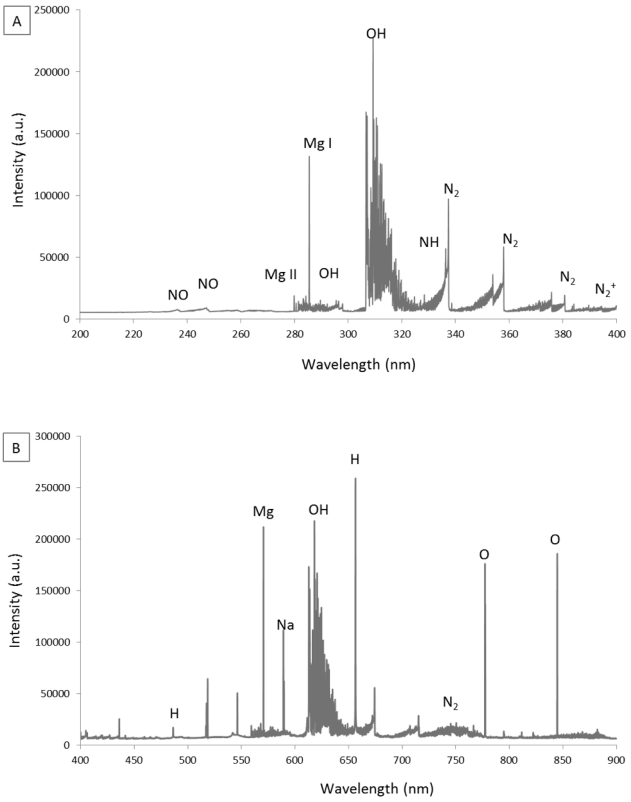

Fig. 2. OES spectra acquired during synthesis of PAL.

pH and conductivity of the PAL are summarized in Table 1 and compared to the PAL precursor solution. As can been seen, pH of Pal decreased, while its conductivity increased, in relation to the PAL precursor solution. These differences may result from enhanced production of nitric (HNO_3), nitrate (HNO_2) or peroxynitrite (O=NOOH) acids in PAL [1].

Table 1. Physicochemical properties of PAL precursor solution [0.1% (m/m) $MgSO_4$] and PAL.

	pH	Conductivity [μS/cm]
PAL precursor solution	6.735	14.4
PAL	2.901	274

The obtained PAL effectively inhibited the growth of *P. parmentieri* IFB5308 in liquid TSB medium, if the concentration of the post-plasma solution applied exceeded 25%. As presented in Fig. 3., application of PAL diminished the severity of soft rot symptoms caused by *P. parmentieri* IFB5308 on potato slices in a statistically significant manner (Welch t-test, $p < 0.05$).

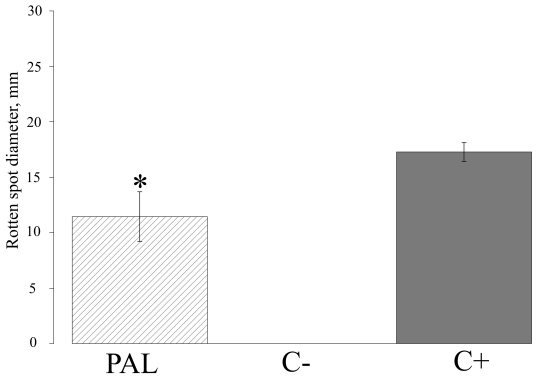

Fig. 3. The effect of PAL on soft rot symptoms caused by *P. parmentieri* IFB5308 on potato slices. PAL – slices were treated with the bacterial solution and PAL, C- – negative control; slices were treated with physiological saline, C+ – positive control; slices were treated with the bacterial solution solely.

4. Conclusions

Within the presented work, it was found that utilization of the dc-APGD–based continuous-flow reaction-discharge system is a promising alternative to stationary systems reported in the literature, in which PALs are synthesized. The presence of reactive individuals in PAL is putatively responsible for its antibacterial properties, which were demonstrated against the plant pathogenic *P. parmentieri* IFB5308 strain, both in liquid culture and within potato tissue. Our results suggest that PAL might be efficiently used for eradication of plant pathogenic microorganisms, but whether is shall be applied. Its applications in a form of fog, spray or watering, needs further research.

5. References

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