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Characterising the impact of post-treatment storage on chemistry and antimicrobial properties of plasma treated water derived from microwave and DBD sources

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The biological effects of atmospheric cold plasma generated reactive species are mediated through and at a liquid interface. The diversity of antimicrobial efficacy or intensity of effects may differ with respect to the plasma device or set up, and it is important to understand how these differences occur to advance understanding and successful applications. Thus, plasma treated water (PTW) from a microwave driven plasma source (PTW-MW) and plasma treated water from a di-electric barrier discharge system (PTW-DBD) were compared in terms of long lived reactive species chemical composition and antimicrobial activity. The influence of a post-treatment storage time (PTST), where reactive species in the gas phase were maintained in contact with the liquid was investigated. Nitrogen-based chemistry dominated in PTW-MW, with high concentrations of nitrous acid decomposing to nitrite and nitrate, while H_2O_2 and nitrate were predominant in PTW-DBD. PTST could enhance H_2O_2 concentrations in di-electric barrier PTW over time while nitrous acid, the main oxidative species in microwave driven PTW, decreased. This work highlights that plasma treated water presents a resource comprising a range of different compounds, stabilities and reactivities which may be tunable to specific applications.

1 Introduction

Non-thermal atmospheric pressure plasma, referring to an ionized gas in which the free electrons are not at a thermal equilibrium with the heavy gas particles, can be generated using a wide array of different plasma devices and types of discharge. These include atmospheric pressure plasma jets, corona discharge, di-electric barrier discharge (DBD) or microwave (MW) driven plasmas,[1] with the resulting plasma containing charged particles (electrons, positive and negative ions), radicals, excited molecules and photons.[2]

The exposure of water to plasma glow or afterglow introduces chemical changes in the liquid, notably a reduction in pH and an introduction of reactive oxygen (ROS) and/or reactive nitrogen species (RNS), with hydrogen peroxide, nitrite and nitrate being among the most

commonly detected species due to their relative stability.[3, 4] Plasma treated water derives antimicrobial activity from the synergistic effects of the plasma generated reactive species and low pH [5] and has demonstrated potential in decontamination of surfaces or food products [6] as well as having potential for medical applications.[5, 7] While the reduction in pH is essential for antimicrobial effects, it has been demonstrated that neither HNO_3 nor HNO_2 at comparable pH level achieve the same effect.[8, 9] It has been proposed that unstable reactive species such as peroxynitrite or peroxynitrate are responsible for the antimicrobial activity of plasma treated water but a definite identification and proof of effect of these factors has been complicated by their unstable nature.[5, 10] The stability of those factors possessing microbicidal properties appears to be a function of storage time and antimicrobial efficacy is lost within a few days of treatment. Julak et al reported that water exposed to corona discharge retained antimicrobial properties for 4 weeks, however the effect was diminished compared to freshly treated solution.[7]

A range of terminology exists to describe water which has been exposed to plasma discharge: plasma activated water (PAW), plasma processed water (PPW), ozone water. Not only do the vast variety of available plasma technologies and devices generate very different plasma discharges, they can also result in water with distinct properties, as plasma type, device and mode of exposure determine the predominant reactive species chemistry generated and thus retained in plasma treated water. In addition, the modulation of species-specificity in plasma-treated water can be achieved through the use of different working gases[11] or simple modification of the discharge set-up.[12]

In this study plasma treated water, generated through the indirect exposure of water to the discharge of a microwave-driven plasma device developed at the Leibniz Institute for Plasma Science and Technology (INP) in Greifswald, Germany, was compared to water exposed

directly to a DBD-based system developed at Dublin Institute of Technology (DIT), Dublin, Ireland, in terms of chemical composition and antimicrobial properties. While the former device generates predominantly reactive nitrogen species (RNS),[14] the latter is rich in ROS[15] (Table 1). The MW-discharge occurs at high temperatures (400K) producing a gas-phase of NO_2 and other NOx without detectable reactive oxygen species, which is cooled prior to contact with water in a glass bottle. The dielectric barrier discharge system uses an 'in-package' principle of plasma treatment, where the plasma discharge is generated inside a gas-sealed air-filled space and can be retained inside the package over various periods of post-treatment storage time (PTST). The system generates high concentrations of ozone in the headspace of the enclosed treatment package, which decreases to undetectable concentrations within 24 h PTST. [13] Previous studies have shown that this post-treatment storage time of the gas-phase reactive species in contact with samples increases their diffusion into/reaction with the liquid increasing concentrations of certain reactive oxygen species in the treated liquid and enhancing the antimicrobial efficacy.[13] The role of a post-treatment storage time under gas-sealed conditions in modulating the chemical composition and biological activity in particular was investigated for plasma-treated water from a ROS-rich DBD discharge and a RNS-dominated MW-discharge, respectively, and highlights that plasma treated water from different sources has very distinct characteristics.

Table 1: Properties of microwave-driven (MW) plasma device and di-electric barrier discharge (DBD) device.

	MW	**DBD**
Frequency	2.45 GHz	50 Hz
Input power	1.1 kW	300-350 W
Input energy	90 – 920 W min	300-3500 W min
Gas temperature	4000 K	<311K (38°C)
T of water during treatment	ambient	ambient
Gas flow rate	18 slm	none
Electron density	$8 \cdot 10^{18}$ m^{-3}	
Main gas reactive species	NO (6000pm) NO_2 (18000ppm)	O_3 (2000-16000ppm) N_2O_4 (1000ppm) NO_2 (1000ppm)

	HNO_2, HNO_3, CO_2, H_2O (2300ppm) No ozone (plasma-on time 7s)[14]	N_2O_5 (1000ppm) NO_3(10-100ppm) (treatment time 1min, 2min)[15]

2 Experimental Section

2.1 Plasma Sources and treatment

Non-thermal plasma treatment of deionized water was performed using the microwave driven discharge PLexc® (plasma excited device) built at Leibniz Institute for Plasma Science and Technology e. V. (Greifswald, Germany) which has been described in detail.[14] Typical operation data used for this work are shown in Tab. 1.

The torch like discharge was ignited for 5 s, 25 s or 50 s at 2.45 GHz and generated plasma treated air (PTA), which was introduced into a 250 ml laboratory glass bottle (Duran Group GmbH, Mainz, Germany) filled with 10 ml sterile deionized water and generated plasma treated water (PTW-MW) (Fig. 1).

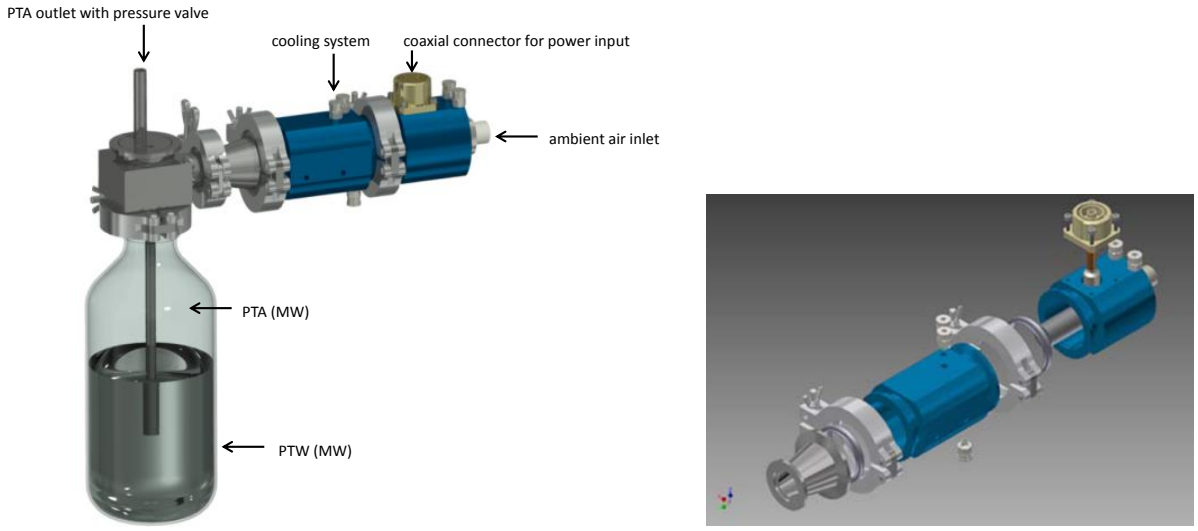


Figure 1. Left: Schematic of the microwave-setup for the generation of plasma treated water (PTW-MW).[16] Right: exploded drawing of the plasma source PLexc[29]

PTW-DBD was generated using a high-voltage di-electric barrier discharge atmospheric cold plasma system custom built at Dublin Institute of Technology (Dublin, Ireland) with a maximum operating voltage of 120 kV_{RMS} at a frequency of 50 Hz which has been described

in detail.[15, 17] Deionized water (10 ml) was placed in the discharge area between high voltage and ground electrode in petri-dishes inside a polypropylene container and sealed with an air-tight film (Cryovac, B2630, USA) and plasma was generated in-package (Fig. 2).

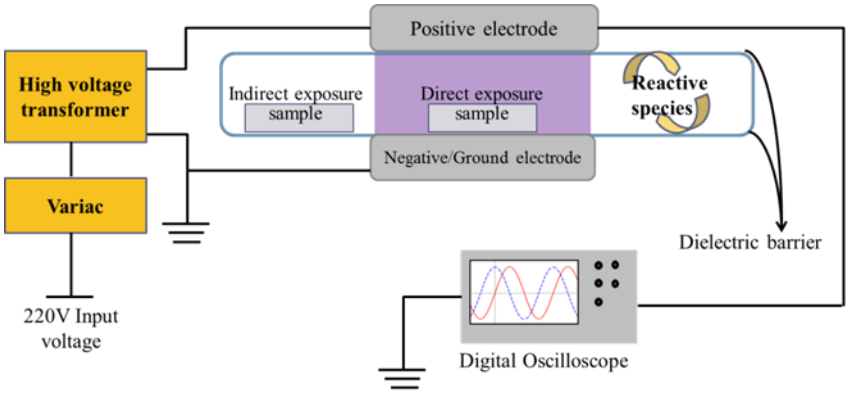


Figure 2. Schematic of the dielectric barrier discharge (DBD)-setup for the generation of plasma treated water (PTW-DBD).[18]

2.2 Post-treatment storage time (PTST)

To investigate the antimicrobial effects and possible changes in liquid analytical parameters (UV spectra, hydrogen peroxide, pH, oxidation reduction potential (ORP), nitrate, nitrite) depending on post-treatment storage time, plasma-treated water was stored in closed bottles (PTW-MW) or inside a gas-sealed box (PTW-DBD) at room temperature for 24 h to allow further diffusion and reaction of gas-reactive species with the liquid. Samples marked as '24h PTST open' were retained in the treatment bottles for 24 h post-treatment but lids were open to allow outward diffusion of gas species. Subsequent storage of all samples was in sample tubes at 4°C.

2.3 Microbiological Investigation

For microbiological investigations three different strains of *Escherichia coli* (DSM 11250, KE 9062, NCTC 12900) were used. Overnight cultures of *E. coli* were prepared in CASO (TSA) broth (Carl Roth, Karlsruhe, Germany and Biokar, Pantin, France) and incubated for 24 h at 37 °C. The overnight cultures were washed thrice with phosphate buffered saline solution

(PBS) and bacterial suspensions were diluted to a working solution of $10^6 – 10^7$ colony forming units per milliliter (CFU/ml).

20 µl of the *E. coli* working solution were pipetted into each well of a 96-well plate and 180 µl plasma-treated water was added respectively. For controls 180 µl untreated water was added. The exposure time of bacteria to treated and un-treated water was 30 s or 60 s. Following the treatment time 60 µl 4.5x PBS was added to neutralize the pH and to stop the reaction. 50 µl of *E. coli* suspension were spiral plated onto CASO (TSA) Agar (Carl Roth, Karlsruhe, Germany and Biokar, Pantin, France) or diluted manually in maximum recovery diluent (MRD) and plated as 10 µl cell suspension spots. The agar plates were incubated for 24 h at 37 °C and the number of viable bacteria were determined by spiral plate count method (Eddy Jet2 IUL, SA, Barcelona, Spain) or manual counting.

To determine the dependency of microbial inactivation on pH, PTW-DBD was neutralized through the stepwise addition of concentrated buffer solution (4.5x PBS), during which the pH was monitored until it had reached neutral. Subsequent re-acidification was through addition of 10mM HCl until the pH had decreased to previous pH of PTW (about 2.7).

2.5 Chemical Analyses

For chemical analyses plasma-treated water without *E. coli* suspension was examined. The spectrophotometric measurements of nitrate, nitrite and UV absorption spectra were performed using a UV/Vis-Scanning-Spectrophotometer (UV-3100PC, VWR, Darmstadt, Germany).

Hydrogen peroxide concentrations were determined using Titanium(IV) oxysulfate sulfuric acid solution (Sigma-Aldrich) by adding 15 µl of $TiOSO_4$ solution to 150 µl of plasma-treated water and the corresponding end-product peroxotitanyl sulfate was measured after 10 min incubation at 405 nm using a Multimode Plate Reader (Thermo Fisher Scientific, Waltham, MA, USA and Biotek, Swindon, UK). A second method utilizing 1 M potassium iodide (KI)

solution enabled the photometrical measurement of the corresponding end-product iodine after 30 min incubation at 390 nm. In brief, 50 µl of phosphate buffer (pH 7) and 100 µl of 1 M KI solution were added to 50 µl of plasma-treated water. Peroxide quantification was achieved through comparison to a standard curve of known hydrogen peroxide concentrations.

The pH and ORP measurements were performed using a multiparameter analysis instrument model inoLab® IDS Multi 9310, a SenTix® MIC pH electrode (pH 0-14/0-100 °C) and a SenTix® ORP electrode (ORP-T 900 0-100 °C; WTW Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany).

Nitrate concentrations were detected using Spectroquant® Nitrate Test (Merck, Darmstadt, Germany) and spectrophotometric measurement at λ = 340 nm after 10 min incubation and compared to a sodium nitrate standard curve. The method is analogous to DIN 38405-9:2011-09.[19]

Nitrite concentrations were determined using Spectroquant® Nitrite Test (Merck KGaA, Darmstadt, Germany) and spectrophotometric measurement at λ = 525 nm after 10 min incubation and compared to a sodium nitrite standard curve. The method is analogous to DIN EN 26777:1993-04.[20] Additionally, nitrite analysis was performed using Griess reagent and measurement at 548 nm.

2.6 Statistical Analysis

Data are presented as mean and standard deviation of triplicate measurements where applicable unless number of replicates n is specified. Data analysis including ANOVA was performed using Graph Pad Prism.

2.7 Abbreviations and nomenclature used

Table 2: Description of abbreviations and nomenclature used.

PTW	Plasma treated water
MW	Microwave plasma
DBD	Dielectric barrier discharge plasma
PTW-MW	Plasma treated water generated by microwave discharge
PTW-DBD	Plasma treated water generated by dielectric barrier discharge

PTST (closed)	Post-treatment storage time during which gas reactive species remain in contact with the liquid in a closed treatment container
PTST open	Post-treatment storage time during which water is held in the treatment container, which is open to allow outward diffusion of gas species
Storage time	Storage of plasma treated water in sample tubes at 4°C

3 Results and Discussion

3.1 PTW of microwave driven discharge

3.1.1 Chemical composition

Post-treatment storage time showed little influence on ORP and pH of the PTW. The ORP increased from around 400 mV in untreated samples to 600-700 mV in plasma treated water, and was slightly higher in samples exposed to PTST (Fig. 3). The pH decreased from neutral to acidic (1.5 – 2) without significant differences resulting from the storage time.

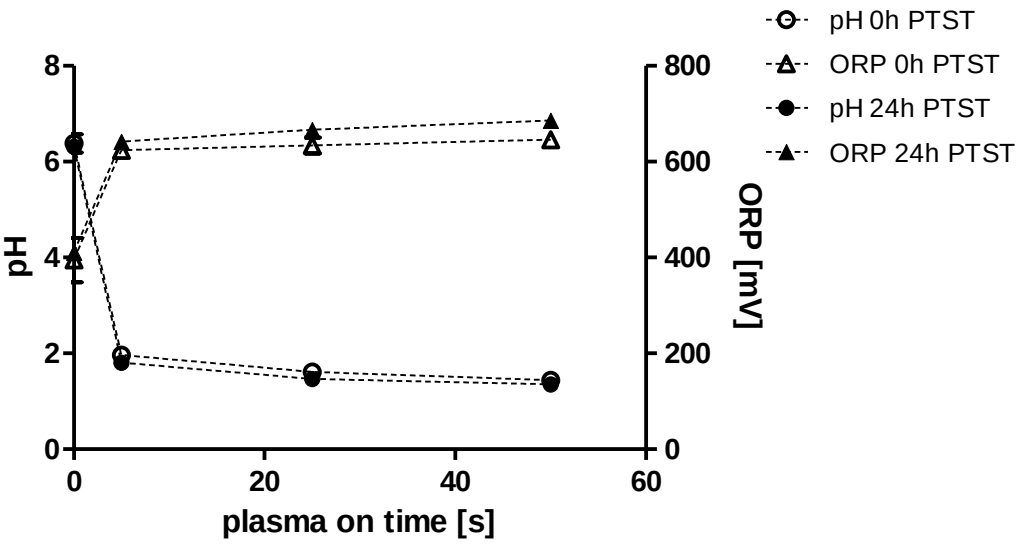


Figure 3. The effect of PTST on PTW pH (circles) and ORP (triangles). PTW-MW was transferred and measured immediately (0 h PTST, open icons) or left under closed conditions for 24 h PTST (closed icons) at room temperature. n = 15 (0 h), 12 (24 h).

Based on measurements using $TiOSO_4$, the PTW-MW did not contain detectable concentrations of H_2O_2 in fresh samples or in samples with 24 h PTST. $TiOSO_4$ is a detection method specific for H_2O_2 and does not react with other reactive species including nitrate and nitrite. Potassium iodide reacts with oxidative species, comprising H_2O_2, to yellow iodine,

which can be quantified spectrophotometrically, but reacts with neither nitrate nor nitrite ions. PTW showed very high reactivity with KI when assayed immediately after processing. When expressed in H_2O_2 equivalent derived from a H_2O_2 standard curve, the concentrations increased in a treatment-time dependent manner and reached concentrations up to an equivalent of 12 mM H_2O_2 for a plasma-on time of 50 s. Re-evaluation of KI reactivity 24 h later in samples stored at 4°C showed some loss of reactivity, particularly in those samples with high concentrations of reactive species. However, concentrations remained at least an order of magnitude above levels determined in samples with 24 h (closed) PTST at room temperature (Fig. 4).

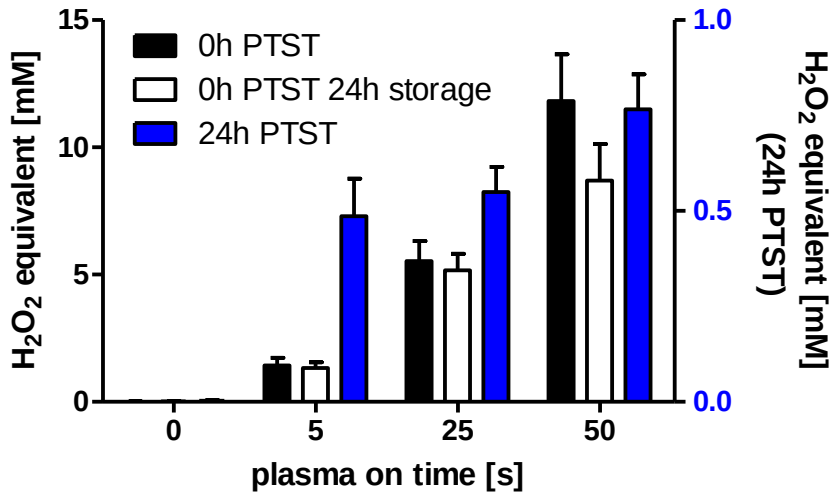


Figure 4. The effect of PTST on reactive oxygen species of PTW. PTW-MW was transferred and measured immediately (0h PTST), measured again after 24 h (0h PTST, 24h storage), or left under closed conditions for 24 h PTST before analysis.

The microwave driven plasma system used in this study generates NO_2 in high concentrations which is visible as a brown gas inside the glass bottles and has been identified as the predominant species using MS in previous studies.[14] The colour dissipates within minutes of storage time in closed containers indicating dissolution of NO_2 into the liquid and/or further reactions. UV spectrophotometry showed very intense peaks for nitrogen containing species

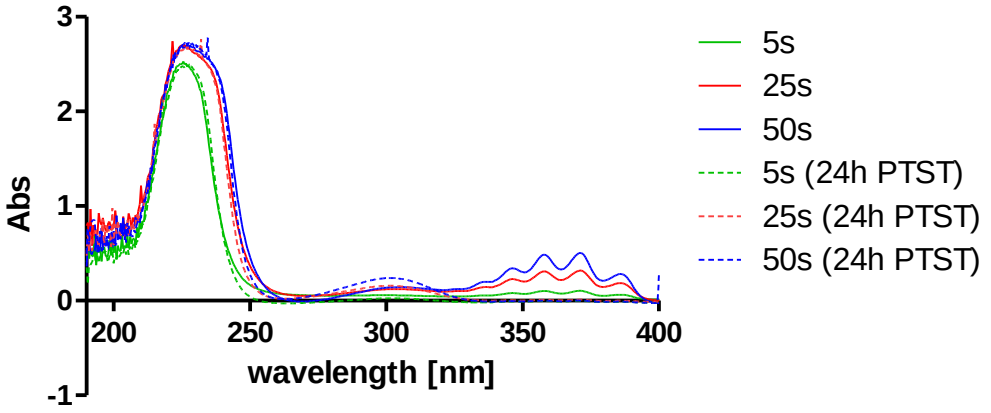
around 200 nm (Fig. 5a) and the characteristic 5 peak "fingers" between 300 and 390 nm (Fig. 5b) identified one of the predominant reactive species in fresh PTW-MW as nitrous acid.[21] Dilute nitrous acid is able to oxidize I^- to I_2, while dilute nitric acid cannot, despite being a more powerful oxidizing agent, and is therefore the likely species which reacts with KI in the absence of H_2O_2. With the exception of very dilute, cold solutions, nitrous acid rapidly decomposes into nitrogen dioxide, nitric oxide, and water, with nitrogen dioxide disproportionating into nitric acid and nitrous acid. The overall reaction of nitrous acid amounts to the production of nitric acid, water, and nitric oxide in warm or concentrated solutions (eq 3):[22, 23]

$2\ HNO_2 \rightarrow NO_2 + NO + H_2O$ (1) $\quad k_1 = 5\text{-}25\ M^{-1}\ s^{-1}$

$2\ NO_2 + H_2O \rightarrow H^+ + NO_3^- + HNO_2$ (2) $\quad k_2 = 5\text{-}10 \times 10^7\ M^{-1}\ s^{-1}$

$3\ HNO_2 \rightarrow H^+ + NO_3^- + 2\ NO + H_2O$ (3) $\quad k_3 = 0.1\text{-}3 \times 10^{-6}\ M^{-1}\ s^{-1}$ [23]

Decomposition of HNO_2 to HNO_3 explains why pH values remained insignificantly changed after the 24h storage period and furthermore explains a rapid loss of reactivity of PTW-MW with KI when diluted with buffered solution and after storage at room temperature.



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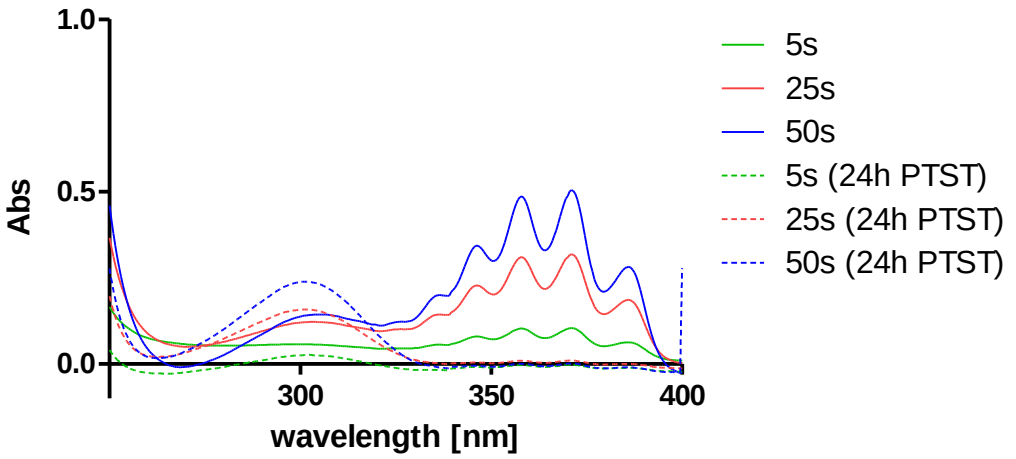

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Figure 5 a,b. UV spectra of PTW-MW generated using microwave discharge (overview spectra 190-400nm (a), detailed spectra 250-400nm (b)).

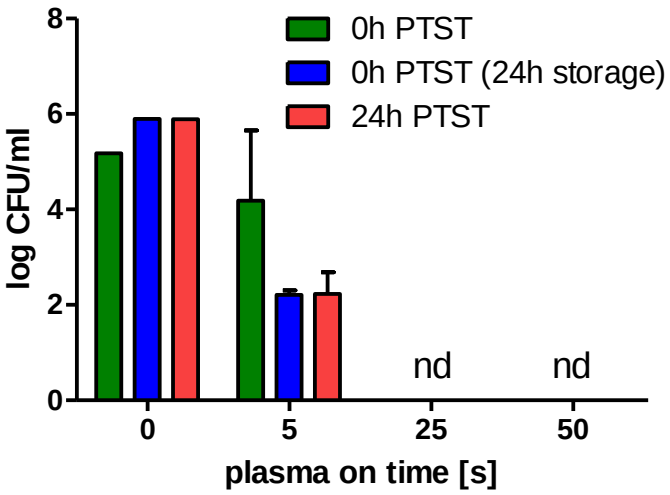
Tatarova et al detected HNO_2 in the exhaust gas stream of a microwave plasma working with air-water mixtures by MS and FTIR analysis.[24] The generation of nitrites in aqueous solution which has been reported for various plasma sources, results in nitrous acid formation at a pH below 3.5 in non-buffered solutions. Decomposition reactions of HNO_2 can form nitrosonium ions or nitrogen radicals such as $NO\cdot$ and $NO_2\cdot$ with strong cell toxic activity.[9]

3.1.2 Antimicrobial activity

The plasma treated water showed very strong antimicrobial activity and a 60 s exposure of *E. coli* to PTW with a plasma-on time of 25 or 50 s was able to decrease concentrations below the detection limit, while up to 3 $\log_{10}$ cycles reduction was obtained by the 5 s-PTW-MW (Fig. 6a). Buffering of the PTW-MW to a neutral pH resulted in a complete loss of its antimicrobial activity (results not shown).

The reaction of KI with PTW in the absence of H_2O_2 serves as an indicator of other reactive oxygen species in the liquid. Microbial inactivation rates of all three *E. coli* strains tested, did not, however, reflect the differences observed in reactive species concentrations. Microbial concentrations were undetectable for plasma treatment above 25 s regardless of the 10 fold

difference in detected oxidative species in PTW-MW analysed immediately post-treatment or stored for 24 h (Fig. 6b). It can therefore be inferred that these species are not immediately or critically responsible for the antimicrobial activity. Previous work by Schnabel et al investigated the effect of HNO_3 in comparison to PTW-MW for microbial inactivation.[8] However, in view of the identification of high concentrations of nitrous acid, a comparison to HNO_2 would be more pertinent. Antimicrobial efficacy of PTW-MW did not reflect the drastic changes in chemical composition and implies that nitrous acid or acidified nitrite *per se* did not represent the main microbicidal species. Free nitrous acid (protonated nitrite) has shown inhibitory activity on a range of microorganisms and has the ability to form derivatives, including NO, NO_2, N_2O_3, a number of which can easily cross cell membranes and are cell toxic.[25, 26] While antimicrobial activity of freshly treated PTW-MW may thus result from free nitrous acid and its immediate decomposition products, more stable derivatives should be responsible for the toxic effects of PTW-MW after storage.



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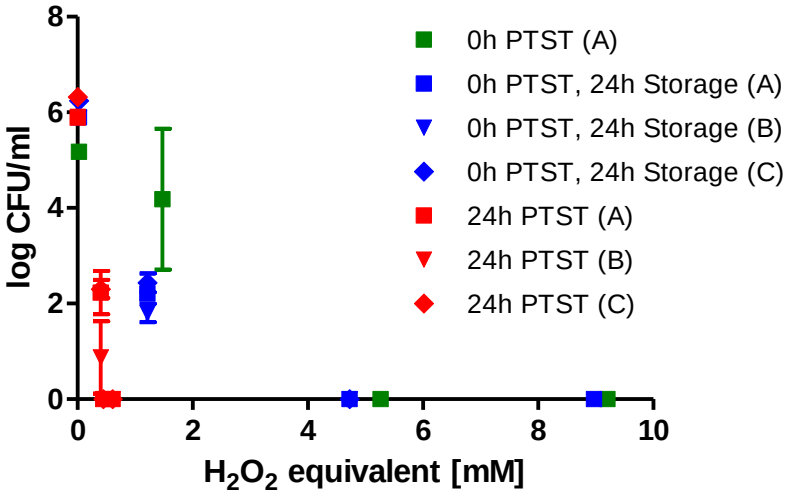

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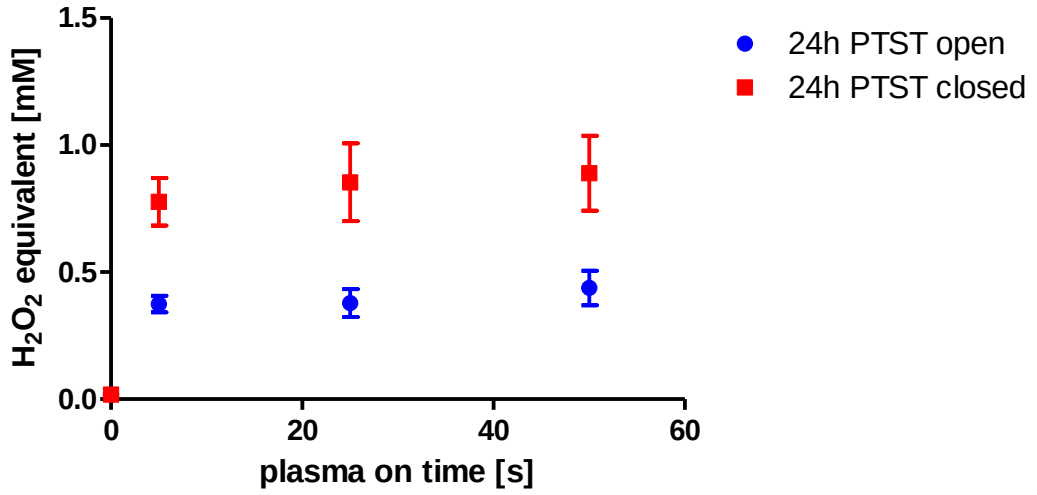
Figure 6 a,b. Microbial inactivation as a function of plasma-on time for *E. coli* DSM 11250 (A) (a) or concentration of H_2O_2 equivalent in PTW-MW (b) for *E. coli* (DSM 11250 (A), KE 9062 (B) and NCTC 12900 (C)) exposed to PTW for 60 s. **PTST** refers to post-treatment storage time in a closed container, **Storage** refers to storage of PTW after transfer into sample tubes. nd = not detected.

3.1.3 Chemical composition: Comparison of PTST between open and closed containers

To discount effects in species composition due to potential degradation during the 24 h storage period post-treatment, direct comparisons were undertaken between PTW-MW that had been stored for 24 h in a bottle closed directly after its filling with plasma treated air, allowing for extended contact time between reactive species in the air and the water; or stored in an open bottle, where gas-reactive species could diffuse out of the container.

Comparison of the reactive species in open and closed containers after 24 h storage at room temperature showed that concentrations in water samples stored under closed conditions were approximately twice as high as those in opened samples. A strong increase of oxidative species after 5 s of plasma-on time was observed but very little effect of subsequent increases of treatment time was apparent (Fig. 7a). ORP and pH levels were comparable to previous measurements and did not differ between the two conditions (Fig. 7b). An equilibrium of nitrous acid with its gas-phase decomposition products NO and NO_2 could be the cause of the higher KI reactivity in closed containers whereas diffusion of these species from open bottles

would further promote nitrous acid decomposition. Comparison of nitrite concentrations after storage to those detected immediately post treatment showed decomposition of nitrite from initial levels of 2-20 mM down to 0.1 mM in samples stored under open conditions and around 0.15 mM in closed samples (Fig. 7c). The Griess reagent used for nitrite quantification detects both nitrite and nitrous acid while potassium iodide, which indicated concentrations up to 12 mM, does not react with nitrite ions. Nitrate, a stable endproduct of many reactive nitrogen species, showed a very clear dependency on treatment time, increasing from 20 mM after 5 s up to 50 mM after 50 s and concentrations in closed samples were 10-20 % higher than those in open samples (Fig. 7d). The offset of stored samples against initial concentrations supports the generation of nitric acid/nitrate due to decomposition of nitrous acid/nitrite observed in the decrease of nitrite concentrations, where the decrease in molar concentration of nitrite corresponds with the increase in nitrate.



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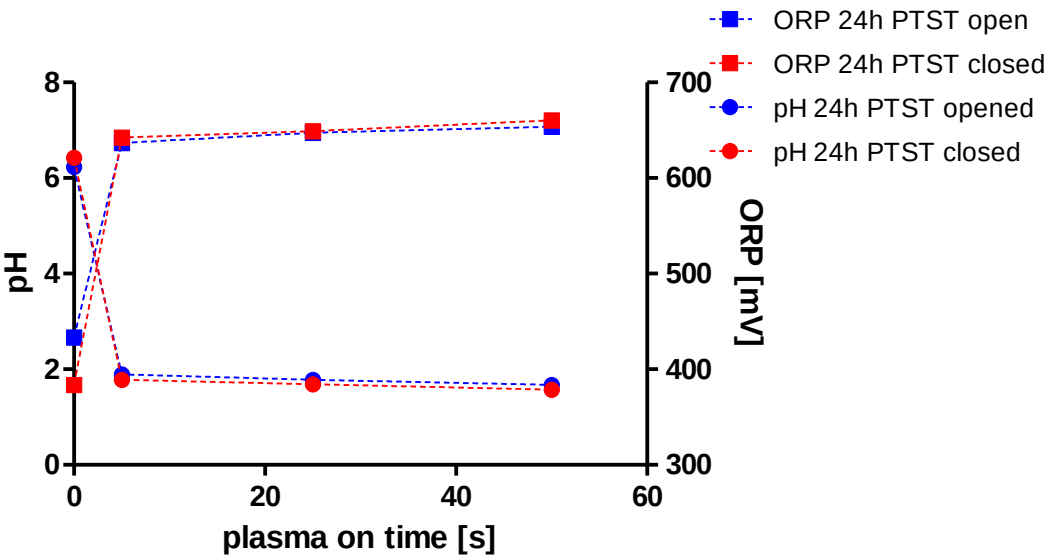

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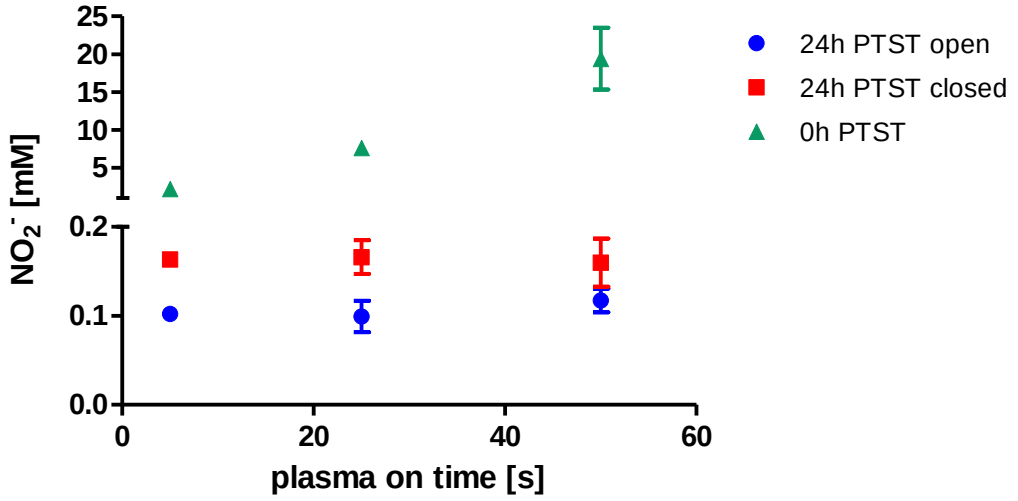

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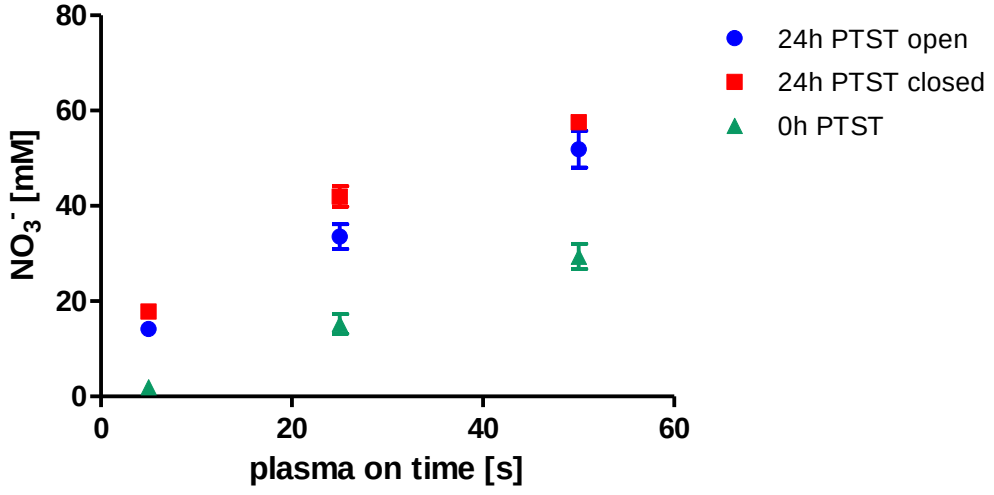

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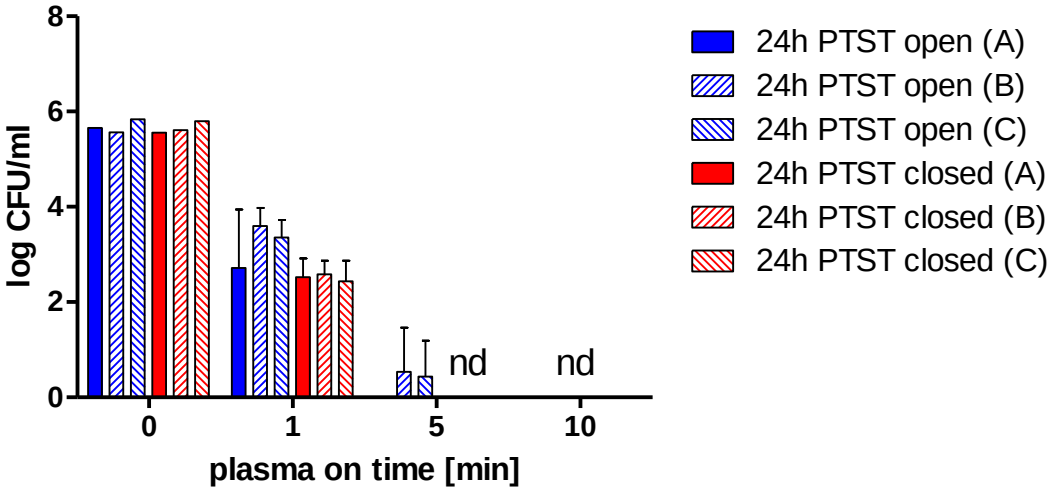
Figure 7 a-d. The effect of PTST in open or closed conditions on pH, ORP (a), oxidative species (b), nitrite (c) and nitrate (d) concentrations (n = 3) compared to samples analysed directly post plasma exposure (0 h).

Despite concentrations of KI-reactive oxidative species being twice as high, similar microbial inactivation was achieved for the three different strains of *E. coli* when exposed to water stored in closed containers compared to open ones (Figure 8a, b)



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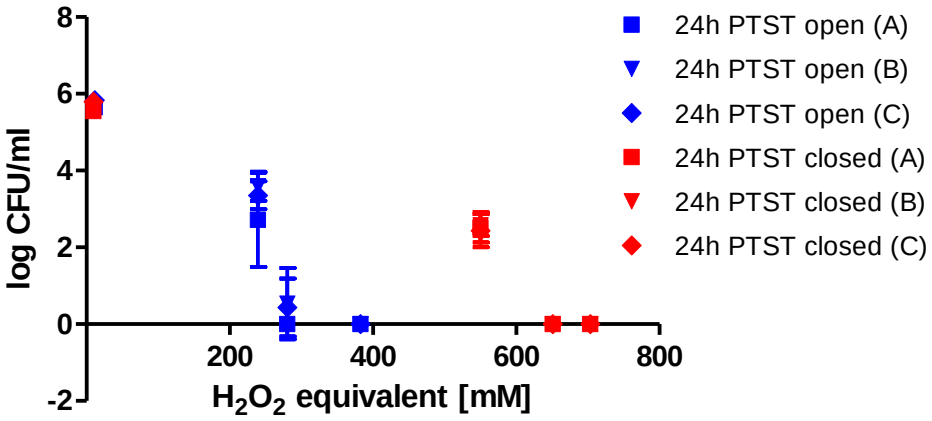

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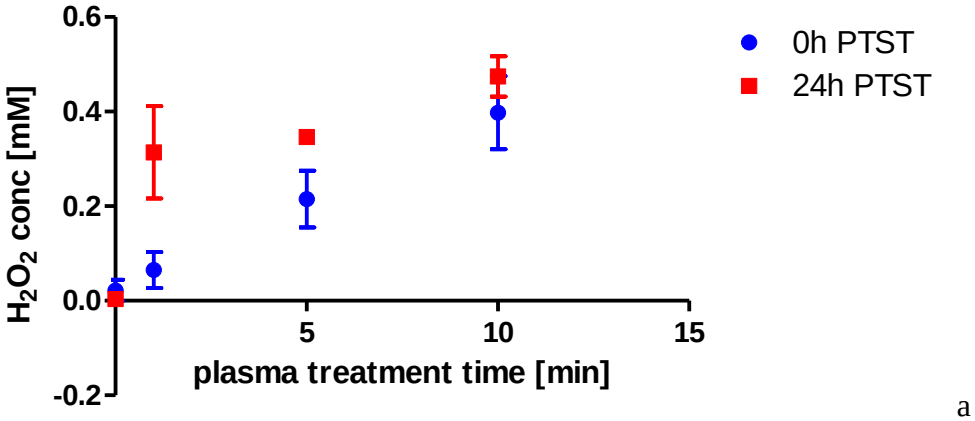
Figure 8a, b. Microbial inactivation as a function of plasma on time (a) and as a function of H_2O_2 equivalent in PTW-MW (b) for *E. coli* (DSM 11250 (A), KE9062 (B) and NCTC 12900 (C)) exposed to PTW for 30 s. nd = not detected.

3.2 PTW of DBD discharge

3.2.1 Chemical composition

In contrast to the PTW-MW described above, PTW generated in-package using a DBD device contained high concentrations of H_2O_2 which increased with PTST (Fig. 9a). $TiOSO_4$ and KI

detection methods yielded results which differed only slightly and suggest little KI-reactive species beyond H_2O_2 to be present in the plasma treated water derived from the DBD system (Fig. 9b).



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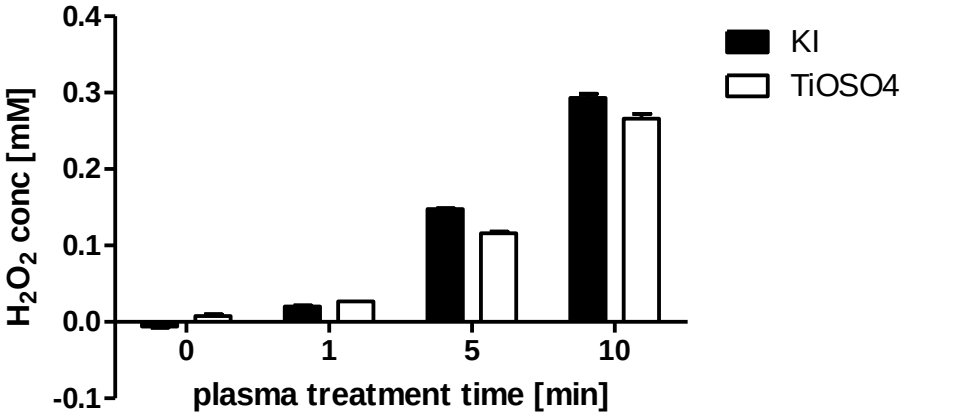

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Figure 9 a,b. H_2O_2 concentrations of different water samples exposed to plasma discharge using a DBD discharge with or without post-treatment storage time, analysed by KI (a) and comparison of measurements using KI and $TiOSO_4$ detection methods for samples without PTST (b).

Nitrate was detected in concentrations below 1mM (Fig. 10) – concentrations 30-50 times lower than those in PTW-MW and not sufficiently high to show discernible peaks at 300 nm (Fig. 11b). UV spectra of PTW-DBD generated at different treatment times showed spectral peaks around 200 nm characteristic of nitrogen compounds but did not indicate any specific nitrite and nitrate peaks at 300 and 350 nm respectively (Fig. 11a, b). The absence of a nitrite peak is in agreement with data obtained from the Griess assay, indicating that nitrite is not

present at detectable concentrations in the PTW-DBD samples. The pH of PTW-DBD was slightly higher than that of PTW-MW, decreasing to pH 3.2 – 2.6 with increasing treatment time.

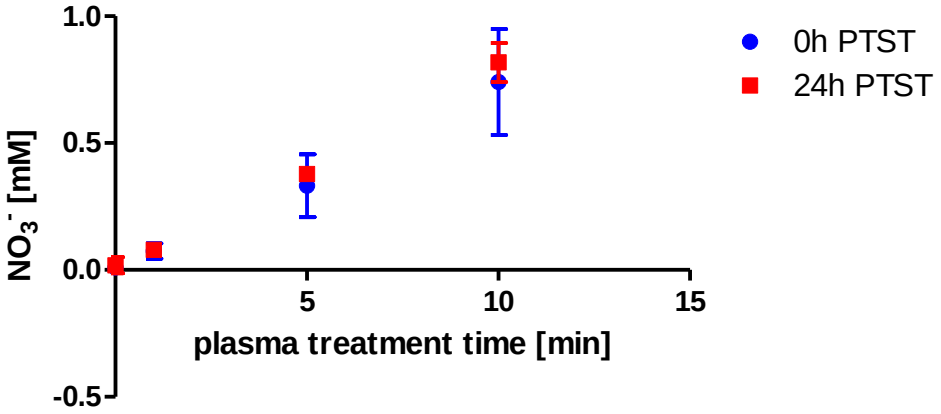
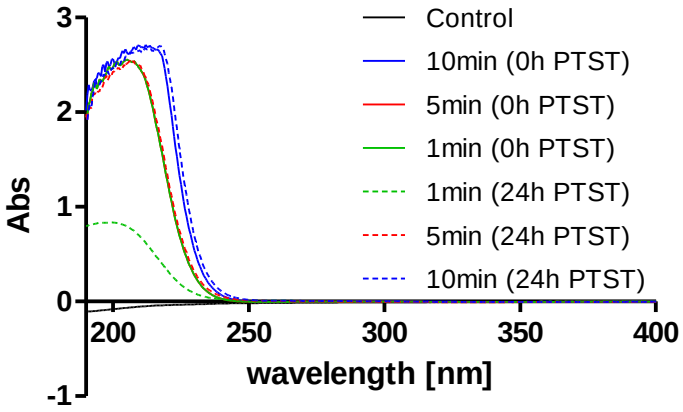


Figure 10. Nitrate concentrations in PTW generated by DBD-ACP with 0 and 24 h PTST.



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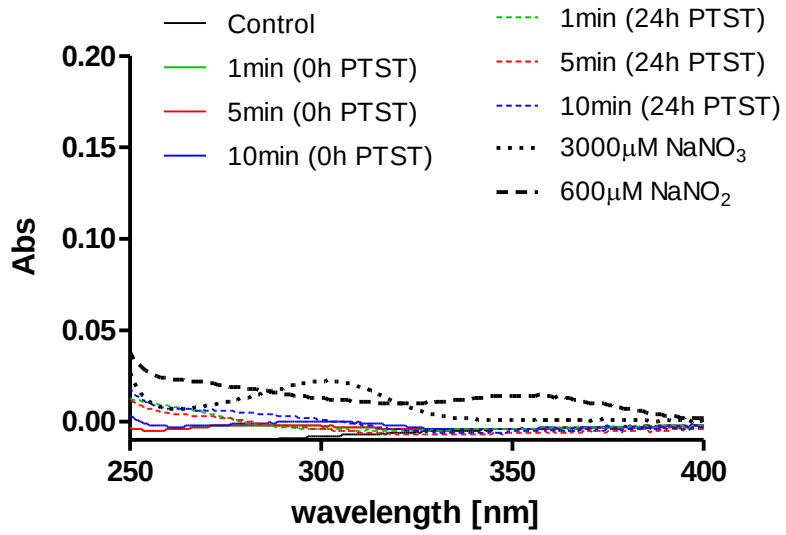

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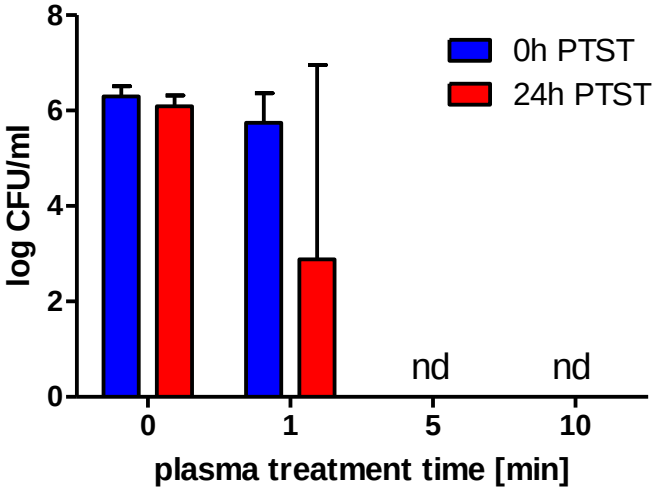
Figure 11a,b. UV spectra of PTW-DBD generated using high voltage DBD discharge (overview spectra 190-400nm (a), detailed spectra 250-400 nm (b)).

3.2.2 Antimicrobial activity

The antimicrobial activity of PTW-DBD was weaker than that of the PTW-MW and longer contact times (1 h) of bacterial suspensions to PTW-DBD were required to achieve inactivation. *E. coli* was undetectable following exposure to PTW-DBD treated for 5 or 10 min with both 0 and 24 h PTST (Fig. 12a). Water treated for 1 min without PTST showed no inactivation capacity. However, including a 24 h PTST had a variable impact on inactivation capacity with effects ranging from complete inactivation to negligible reductions. Thus, while microbial inactivation showed a correlation with H_2O_2 concentrations, the differences observed between samples with and without PTST suggest that this is not the primary causal relationship (Fig. 12b). Microbial inactivation capacity was dependent on the acidic pH of the solution as has been reported by others[8, 9] and was negated through neutralization of the pH. As re-acidification could not re-instate the antimicrobial activity, it can be concluded that the main biocidal factor is acidic in nature and is decomposed at higher pH (Fig. 13). The low pH in itself is not sufficient to inactivate the microorganisms as has been demonstrated for acidified water.[8] Peroxynitrite and peroxynitrate have been suggested as potential biocidal mediators

which could be generated in PTW and decompose to reactive species such as the hydroxyl radical, nitrogen dioxide and singlet oxygen, resulting in microbial inactivation through oxidative damage.[27] Direct detection of both species is complicated by their instability and/or overlapping characteristics with other ROS/RNS.[10]



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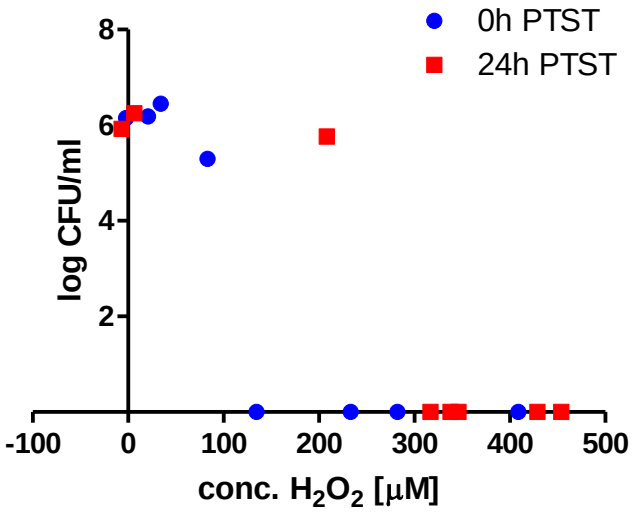


b

Figure 12a,b. Antimicrobial effects of PTW-DBD on *E. coli* (NCTC 12900) exposed to PTW for 1 h dependent on treatment time (a) and corresponding H_2O_2 concentrations (b). nd = not detected.

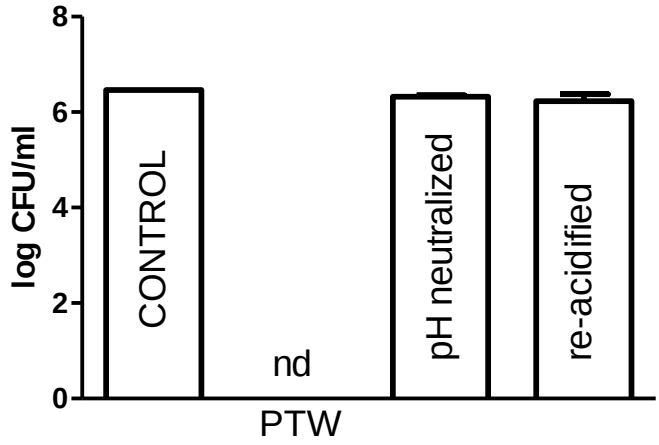


Figure 13. Antimicrobial effects of PTW-DBD on *E. coli* (NCTC 12900) are dependent on the solution's pH. nd = not detected.

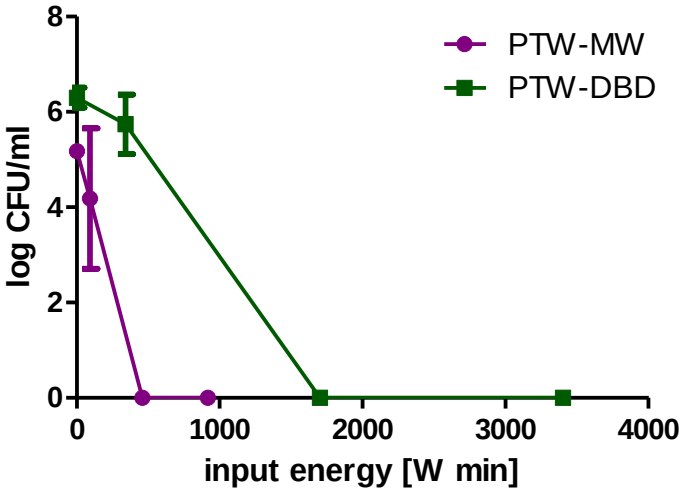
3.3 Comparison of PTW-MW and PTW-DBD

Nitrous acid was identified as a predominant oxidative species in microwave driven discharge generated PTW, which decomposed to give rise to other species including nitrate (Table 3),

In contrast to the PTW-MW, PTW generated in-package from a DBD- source demonstrates a very different chemistry and species evolution over time. Nitrite if present was in undetectable concentrations and nitrate, present at up to 1mM, showed little increase with PTST. H_2O_2 concentrations increased with PTST as a result of the reaction/diffusion of gas-reactive species retained in the sealed package into the liquid and remained stable over time thereafter.[28]

These findings support observations from other plasma sources where nitrite and H_2O_2 generation were exclusive of each other.[12] Work by Lukes and co-workers[9] has demonstrated the pH dependent reaction of H_2O_2 and nitrite to form peroxynitrite, a reaction which could be a cause for the absence of detectable H_2O_2 in PTW-MW and undetectable levels of nitrite in PTW-DBD. However, previous studies detected no $O\cdot$, $O_2\cdot$, $OH\cdot$ or O_3 and indicated a lack of ROS generated by the MW plasma device due to high gas temperatures in the centre of the plasma.[14] For the DBD device which generates high concentrations of H_2O_2, a reaction with nitrite during/directly post discharge is possible and could have resulted in the formation of the detected NO_3^-. Species measurements suggest that even if such reactions took place, RNS

species were largely in excess in the MW-PTW while ROS were in excess in PTW-DBD. The antimicrobial efficacy of PTW-DBD was much lower than its PTW-MW counterpart, requiring contact times in the range of minutes to hours. Comparison of input energy for the two devices with respective treatment times indicated much higher potency of PTW-MW even at lower energy input (Fig. 14a, b). Despite the predominance of distinct reactive species in the two types of water, with nitrous acid/nitrite being predominant in PTW-MW and H_2O_2 in PTW-DBD at similar pH, these solutions behaved similarly with regard to the nature of their antimicrobial effect which was dependent on pH and lost over storage time in a matter of days. The oxidative species measured through reaction with KI, namely nitrous acid and H_2O_2, showed distinct changes with PTST, where the reactivity in PTW-MW decreased with storage time, but increased in PTW-DBD. However, a direct correlation with antimicrobial effects could not be found for either case and a prediction of the plasma treated solutions' efficacy based on its chemical properties was not possible in this study. The major bactericidal activity may be associated with other reactive species distinct from those identified. Both peroxynitrite and peroxynitrate have been implied as potential important bactericidal agents generated in plasma treated aqueous solutions and can be formed through the secondary reaction of ROS and RNS including H_2O_2 and nitrites,[9] while decomposition products of nitrous acid such as NO, NO_2 and N_2O_3 could also be involved.



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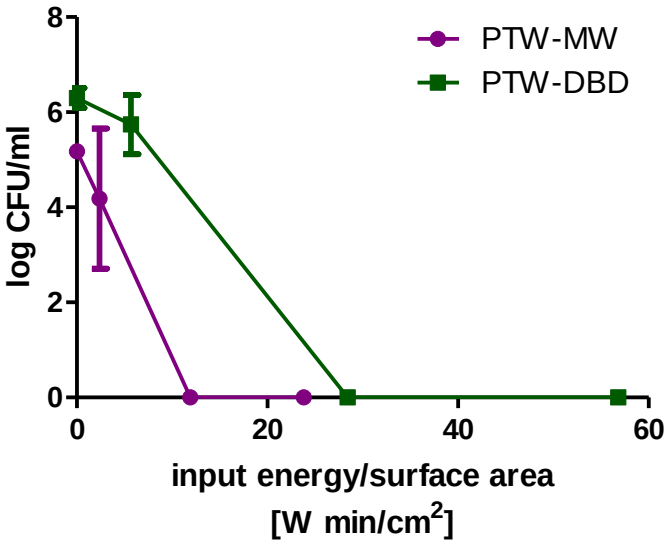

b

Figure 14 a,b. Comparison of antimicrobial effects of PTW-MW and PTW-DBD on *E. coli* for respective input energy (a) and input energy per surface area (b) with a contact time of 1min for PTW-MW and 60 min for PTW-DBD.

Table 3: Comparison of properties and main reactive species composition in PTW-MW and PTW-DBD.

	PTW-MW	**PTW-DBD**
Input energy	90-920 W min	300-3500 W min
Nitrous acid	2-12 mM	Not detected
Nitrite	2-20 mM	Not detected
Nitrate	1-25 mM	0.1-0.8 mM

Hydrogen peroxide	Not detected	0.02-0.4 mM
Contact time for microbial inactivation	~1 min	~60 min

4 Conclusion

This work demonstrates that plasma treated water encompasses a diverse range of reactive species with different chemical and biological effects, with particular reference to their stability over time. The composition of plasma treated water can be influenced by the type of plasma discharge, the working gas composition or the electrode configuration. This diversity means that properties of one type of plasma treated water cannot be generalized and more detailed characterization is required for varying set-ups. It also suggests that plasma treatment of water can be used as a platform for generating a vast range of chemical compounds which can be a resource for diverse chemical and biological applications.

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plasma treated water; PTW

Graphical Abstract

The table of contents entry should be fifty to sixty words long (max. 400 characters), and the first phrase should be bold. The entry should be written in the present tense and impersonal style. The text should be different from the abstract text.

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ToC figure ((Please choose one size: 55 mm broad × 50 mm high **or** 110 mm broad × 20 mm high. Please do not use any other dimensions))

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