



Plasma-treated liquids as an environmentally friendly alternative to conventional disinfectants for the hygienization of food processing facilities – first insights in shelf life characteristics and antibacterial efficacy

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ABSTRACT

Plasma-treated liquids (PTL) are produced using atmospheric pressure plasma and therefore contain reactive nitrogen species and reactive oxygen species. Due to these reactive species, the liquids exhibit an antimicrobial effect. Therefore, their use as disinfectants is being widely investigated, especially for the food industry. However, there are only a few studies on the efficacy of freshly prepared or stored PTLs on their use as surface disinfectants.

In our studies, we produced PTLs from two different base liquids, saline and deionized water, by treatment with an arc discharge plasma jet. The liquids were characterized after production using sum parameters of the reactive species (pH, oxidation-reduction-potential (ORP) and electrical conductivity). Both PTLs were additionally characterized during storage. In microbiological studies, the antibacterial efficacy of fresh PTLs was tested on different surfaces (acrylonitrile butadiene styrene (ABS), polyvinyl chloride (PVC) and stainless steel). Our studies revealed that all PTL types achieve a reduction over 4 log, even though the characteristics differ. The antibacterial efficacy of PTL from saline was additionally evaluated during storage and decreased slightly, which is accompanied by a change in pH, ORP and electrical conductivity during the first days of storage. However, this PTL though showed good antibacterial efficacy after 8 days of storage, by inactivating up to 4.9 log of *E. coli*. This illustrates the potential of PTL to be used as disinfectant in food processing and production.

1. Introduction

The investigation of plasma-treated liquids (PTL) as an alternative to conventional disinfection methods in fields of application such as medicine, food industry and agriculture has steadily increased in the last years (Han et al., 2023; Thirumdas et al., 2018; Zhao et al., 2020; Zhou et al., 2020).

PTLs, often also referred as plasma-activated water (PAW) or plasma-activated liquids (PAL), exhibit an antimicrobial effect due to the reactive oxygen species (ROS) and reactive nitrogen species (RNS) and their oxidative effect, as well as the associated low pH (Tsoukou et al., 2018; Zhao et al., 2021). The reactive species are dissolved either by the direct contact of the plasma of an atmospheric pressure plasma source with the liquid or indirectly via the introduction of plasma gas into the liquid. When radicals and charged particles of the plasma are introduced into liquids, the RNS and ROS from the gas dissolve in the liquid, react with each other and with components of the liquid to form further species.

The kind of reactive species formed depends mainly on the plasma source, the process parameters, the treated liquid, as well as the ambient conditions such as humidity and temperature (Han et al., 2023; Tsoukou et al., 2018; Zhao et al., 2020; Zhao et al., 2021; Zhao et al., 2023; Zhou et al., 2020). The reactive species can be divided into short-lived and long-lived species. It is known that the half-lives of the short-lived reactive species ranges from nanoseconds (e.g. hydroxyl radical) to a few seconds (e.g. nitric oxide), whereas the long-lived reactive species exhibit half-lives from minutes (e.g. ozone) to years (e.g. nitrate) (Thirumdas et al., 2018; Zhao et al., 2021; Zhou et al., 2020).

The use of PTL in the food industry has been investigated intensively in several studies in recent years (Han et al., 2023; Thirumdas et al., 2018; Zhao et al., 2020; Zhao et al., 2023; Zhou et al., 2020). The effect of PTL on planktonic microorganisms and microorganisms adhering to food surfaces was widely investigated (Tan and Karwe, 2021; Thirumdas et al., 2018). Many studies have evaluated the usability of PTL for washing foods such as fruits, vegetables and meat, as well as its use as a storage agent for freezing food, along with an assessment of its impact

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Nomenclature

D	dilution factor, -
N	bacterial concentration, CFU/mL
N ₀	bacterial concentration of the blanks, CFU/mL
N _t	bacterial concentration after PTL incubation, CFU/mL
ORP	oxidation-reduction potential, mV
pH	pondus hydrogenii, -
R	reduction, CFU/mL
R _p	percentage reduction, %
R _l	log reduction, log
V _b	plated volume of bacterial suspensions, mL
V _d	dilution volume, mL
\bar{x}_c	mean value colonies of all agar plates of one sample, CFU
σ	electrical conductivity, mS/cm

on food quality (Han et al., 2023; Patra et al., 2022). It was shown that for these applications PTL has a sanitizing effect on the food, it generates less residues compared to chemicals (Zhao et al., 2023) and does not form carcinogenic end- or by-products (Tan and Karwe, 2021). Also, PTLs are more environmentally friendly compared to most traditional disinfecting chemicals and PTLs are suitable for temperature-sensitive objects (Han et al., 2023; Zhao et al., 2020). The use of PTL has therefore been proposed as an alternative to conventional disinfectants such as chlorine solution, peracetic acid and hydrogen peroxide in the food industry for the disinfection-in-place process (Tan and Karwe, 2021; Zhao et al., 2023). However, besides the research concerned with food surfaces, there are few studies on the effect of PTL on adherent microorganisms and biofilms, or on their use for the disinfection of inorganic surfaces and the influence of the surface material on PTL efficacy. Insights into these questions are of essential need for the evaluation of PTL suitability to be used as disinfectant for food production facilities and food-contact surfaces. Additionally, it is relevant to assess the possibility of PTL storage after production to allow industrial application in large volume with unimpaired antimicrobial efficacy. This demonstrates the need for detailed research on the antimicrobial effects of PTL on surfaces in correlation to the storage time, the material dependency, PTL shelf life and the influence of storage conditions (Thirumdas et al., 2018; Zhao et al., 2020; Zhao et al., 2023).

This study expands our previous study (Steinhäuser et al., 2025). There we showed that the efficacy of freshly prepared S-PTLs depends on the material to be treated, the incubation time, and the volume used, and that it was possible to correlate the antibacterial efficacy of fresh S-PTL to the oxidation-reduction-potential (ORP). The current study examines the applicability of PTLs as surface disinfectants in correlation to PTL storage. Therefore, we produced PTL from different base liquids: deionized water (W-PTL) and saline (S-PTL). Both PTLs were characterized indirectly by using the sum parameters pH, ORP and electrical conductivity. The characterization of PTL is based on sum parameters, since there is a large number of individual reactive species whose quantity and varying half-lives complicate an individual characterization. The sum parameters, especially the ORP, are intended to provide a sufficient approximation to describe the characteristics and efficacy of the PTLs and are an established parameter used in many other studies on PTL (Zhou et al., 2018; Zhao et al., 2020; Shen et al., 2016; Rathore et al., 2021; Wang and Salvi, 2021; Han et al., 2023), allowing a comparability with our study. We investigated the antibacterial efficacy and its dependency on surface material: We applied fresh prepared S-PTL and W-PTL on three different materials using a dried surface contamination of the model microorganism *Escherichia coli* (*E. coli*). Additionally, further investigations on the antibacterial efficacy and its dependence on storage time were investigated with S-PTL. Shelf life

evaluation of both PTLs was done over a period of 21 days via measurement of the sum parameters mentioned above, whereby gas exchange during storage was either allowed or prevented.

2. Material and methods

2.1. Plasma source

The production of PTL using the atmospheric pressure plasma system PW-15 (Plasmatrete, Steinhagen, Germany) has already been described in our previous study (Steinhäuser et al., 2025). In this system, the gas from the plasma source is fed into the liquid to produce PTL, thereby avoiding direct contact between the plasma and the liquid. Therefore, it consists of an arc discharge plasma source PF10 (Openair-Plasma, Plasmatrete), a 20 kV high-voltage supply, a heat sink for gas cooling and a column for liquid treatment with a sintered body for gas distribution (Fig. 1). The plasma source, the high-voltage power supply, and the cooling system are located in the plasma system cabinet and are controlled and monitored via a panel attached to the cabinet which assured constant parameters for gas flow, voltage, current and frequency during PTL treatment. The plasma source was operated with dry compressed air. After production, the PTL was purged at the bottom of the liquid column.

2.2. Production of PTL

For comparing the effectiveness of different PTLs, deionized water and saline (137 mM NaCl (Ph. Eur., USP, Carl Roth, Karlsruhe, Germany), 2.7 mM KCl (CELLPURE, Carl Roth)) were used as base liquids for PTL production. Saline was chosen to ensure comparability with our previous investigations (Steinhäuser et al., 2025). Prior to plasma treatment, all liquids were sterilized by autoclaving.

To produce PTL, 2 L of saline or deionized water respectively were filled into the column and treated for 20 min with plasma gas with the following parameters: gas flow: 31–32 L/min, voltage: 278–279 V, current: 10.1–10.4 A and excitation frequency of the plasma jet: 21.6 kHz. The adherence to this consistent process parameters ensured production of the PTLs with reproducible characteristics.

After production, the PTL was immediately aliquoted and stored in several sterile glass laboratory bottles to allow separation for an independent use in microbiological evaluation and shelf life experiments. The bottles were wrapped in aluminum foil and stored in the dark at room temperature.

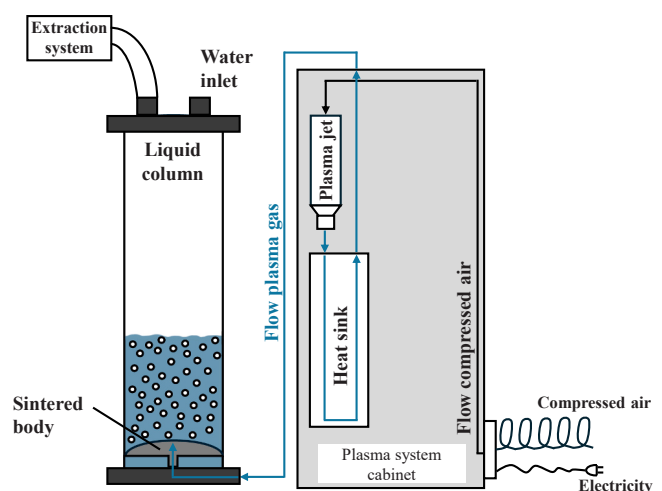


Fig. 1. Schematic drawing of the arc discharge plasma system for PTL production. The illustration is adapted from Steinhäuser et al. (2025).

2.3. Measurement of PTLs and PTL shelf life

The characteristics of the PTLs and PTL shelf life were monitored indirectly as previously described by Steinhäuser et al. (2025) by measuring the ORP, the pH and the electrical conductivity (σ). A multimeter (Orion Versa Star Pro, Thermo Fisher Scientific, Waltham, USA) with an iodine/iodide electrode (Orion ROSS Ultra pH/ATC Triode 8302 BNUMD, Thermo Fisher Scientific) and an electrical conductivity electrode (Orion DuraProbe 013005MD, Thermo Fisher Scientific) was used for this purpose. All ORP values stated in this study were calculated based on the potential of the standard hydrogen electrode.

For shelf life experiments in conjunction with microbiological assessments, S-PTL was used based on our decision in our previous paper (Steinhäuser et al., 2025) and on the results of Tsoukou et al. (2018). They showed that PTL from saline had the highest antibacterial efficacy compared to PTL from other liquids such as distilled water (Tsoukou et al., 2018). The reported higher efficacy of fresh S-PTL and the observed higher change in pH, ORP and electrical conductivity (see Section 3.1), which could be related to the amount of present reactive species, led us to expect that S-PTL would also maintain superior and longer-lasting antibacterial efficacy during storage. Therefore, shelf life experiments on W-PTL were conducted without parallel microbiological experiments.

The pH, electrical conductivity and ORP of W-PTL and S-PTL were measured over a time period of 21 days, in intervals of minutes to hours (directly after production) and then in intervals of days. To investigate the influence of gas exchange on the PTL characteristics during storage, one laboratory bottle with PTL was measured continuously, allowing gas exchange with the ambient air during each measurement for the time of measurement. Additionally, we used for selected time points of measurement (3, 8 and 21 days after production) unopened bottles of PTL, that were kept closed after production until time of measurement. In this way a continuous gas exchange with ambient air was prevented. In addition, an untreated reference liquid of pure sterile saline or pure sterile deionized water was included for the continuous measurement. As measurement points, we chose: 1 min (directly at the end of plasma treatment), 3, 5, 10, 15, 20, 30, 40, 50, 60, 90, 120 and 180 min after production. Then, every next measurement was carried out with intervals of 1–6 d up to 21 days after production (detailed measurement points see Fig. 4).

2.4. Microbiological evaluation

The methods used for microbiological evaluation are summarized in the following sections, detailed explanations can be found in Steinhäuser et al. (2025).

2.4.1. Cultivation and preparation of *E. coli*

The stock culture of *E. coli* K12 (DSM 498) was grown in 100 mL of standard I nutrient broth (15 g/L peptone, 3 g/L yeast extract, 6 g/L sodium chloride, 1 g/L glucose, Carl Roth) for 24 h at 37 °C in a shaking incubator with 125 rpm. To avoid unwanted reactions between the growth medium and the PTLs, 30 mL of the stock culture were purified twice in 30 mL saline by centrifugal separation (10 min at 3000 g).

The bacterial density of the purified cultures was determined with a Neubauer counting chamber (Neubauer-Improved, Paul Marienfeld, Lauda-Königshofen, Germany) by counting the number of apparent bacteria under a light microscope (B3 professional series, MoticEurope, Barcelona, Spain). Based on the calculated bacterial density, the purified cultures were diluted with saline to a final concentration of $2\text{--}3 \times 10^7$ bacteria per mL and used for subsequent sample inoculation.

2.4.2. Sample preparation

To investigate the antibacterial efficacy of PTL, microbiological tests were conducted on 5×5 cm sample pieces of three materials: acrylonitrile butadiene styrene (ABS), polyvinyl chloride (PVC) and brushed

stainless steel. Materials were selected according to our previous studies (Steinhäuser et al., 2025). After surface cleaning with 100 % 2-propanol (VWR International, Radnor, USA), the samples were sterilized for 3 min in 70 % ethanol (Carl Roth) in an ultrasonic bath (45 kHz, 80 W, VWR). After sterilization, all samples were stored in a sterile petri dish for further processing. For inoculation of the surfaces with bacteria, four samples of each material were inoculated with the prepared bacterial suspension in a scheme of 20 drops of a volume of 50 μ L each. The inoculum was allowed to dry for approx. 2 h at room temperature in a sterile workbench, until the inoculated samples showed no residues of moisture. Hence, microbiological experiments started directly after drying. Taking the desiccation stress into account, the preparation of the samples after this protocol allows between 0.5×10^4 and 1×10^5 viable bacteria per sample to be preserved on the surface after drying.

2.4.3. Antibacterial efficacy

To evaluate the antibacterial efficacy of the PTLs, the type of PTL and the storage time was varied, PTL types were designated as follows: fresh prepared (“fresh W-PTL” and “fresh S-PTL”), 3 days of storage (“S-PTL-3d”) and 8 days of storage (“S-PTL-8d”). As described in Section 2.3, microbiological evaluations on stored PTL were only conducted with S-PTL without gas exchange. Each PTL was examined in triplicate in 3 independent experiments and on all materials.

The inoculated samples were incubated for 5 min with 5 mL PTL, whereby the PTL was carefully pipetted on the inoculated area of the samples, resulting in a complete coverage of this area. This step was followed by a 5 min incubation of the sample in the closed petri dish at room temperature. Afterwards, this liquid was completely recovered from the sample with a pipette, filled into a test tube and mixed to allow homogenization of the liquid. Aliquots were then subsequently plated on nutrient agar and incubated as described in Section 2.4.4.

To calculate the bacterial reduction induced by PTL incubation, blank samples were used for each material. The blanks were processed with the same regimes as described above in this section, except that sterile saline or sterile deionized water were used instead of PTL for incubation: The inoculated samples were incubated for 5 min with 5 mL sterile saline or sterile deionized water, whereby the liquid was carefully pipetted on the inoculated area until it was completely covered. After 5 min incubation at room temperature in the closed petri dish, this liquid was completely recovered with a pipette, filled into a test tube and mixed. Aliquots of these recovered liquids were plated undiluted and diluted on nutrient agar and incubated as described in Section 2.4.4.

2.4.4. Plate count method

The number of viable bacteria was determined by the plate count method. For each sample and for the blanks, 100 μ L of the recovered liquid was plated in triplicate on standard I nutrient agar plates (15 g/L peptone, 3 g/L yeast extract, 6 g/L sodium chloride, 1 g/L glucose, 12 g/L agar, Carl Roth). For the blanks, the recovered liquid was plated each in triplicate on nutrient agar plates undiluted, 1:10 and 1:100 diluted with nutrient medium. As positive control, 100 μ L of the prepared bacterial suspension was diluted to 10^3 bacteria per mL and plated on nutrient agar in triplicate. As negative controls, 100 μ L of sterile nutrient medium, sterile saline or sterile deionized water and the PTLs were plated on nutrient agar. Pure nutrient agar was used as a further control to ensure sterility of the used materials and media. All agar plates were incubated at 27 °C under ambient air and 70 % humidity for 15 h, followed by 5 h at 37 °C under ambient air and 70 % humidity. Afterwards, the number of colony forming units (CFU) was determined by optical count.

2.5. Calculation and statistics

For calculation of the mean values and standard deviations (SD), presented as mean \pm SD, Microsoft 365 Excel (version 2507) was used. The following formula was used to calculate the number of viable

bacteria after sample incubation with PTL:

$$N = ((x_c^- * D) / V_b) * V_d \quad (1)$$

The reduction of bacteria by PTL incubation was calculated with the following formulas:

$$R = (N_0 / N_t) \quad (2)$$

$$R_l = \log_{10}(N_0 / N_t) \quad (3)$$

$$R_p = (N_t / (N_0 * 100)) \quad (4)$$

The statistical significance of the data was analyzed with statistics software (GraphPad Prism 6, version 6.01). At first, outliers were excluded (ROUT, $Q = 5\%$) for each data set ($n \geq 3$). Afterwards, the data were checked for Gaussian distribution (Kolmogorov/Smirnov test, Lilliefors test: p-value). Statistical significance was tested using an unpaired, two-tailed T-test (Welch's correlation with 95 % confidence level), if the data were normally distributed. The unpaired, two-tailed Mann-Whitney test with a confidence level of 95 % was used, if one or both data sets were not normally distributed, or it could not be tested. An * marks statistically significant different data ($p < 0.05$).

3. Results and discussion

3.1. Characteristics of fresh PTL

The characteristics of W-PTL and S-PTL immediately after production compared to the corresponding untreated base fluids are shown in Fig. 2 for ORP and pH. The initial values of both base fluids are comparable, with a pH of 5.4 for deionized water and 5.6 for saline, and an ORP of 493.4 mV and 489.0 mV, respectively. Plasma treatment reduces the pH of S-PTL to 2.5 and of W-PTL to 2.6, while the ORP rises to 690.7 mV and 656.4 mV, respectively. The slightly higher changes in pH and ORP of S-PTL show a statistically significant difference to the measured values of W-PTL. The low standard deviations show that PTLs with reproducible characteristics can be produced by plasma treatment of both base liquids.

It is described in literature that during PTL production pH decreases, whereas ORP and electrical conductivity increase, confirming our previous findings (Tsoukou et al., 2018; Wang and Salvi, 2021; Zhou et al., 2018; Steinhäuser et al., 2025). We observed the same correlations in our current study when treating saline and deionized water by plasma.

Due to the production of our PTLs via the indirect plasma treatment method and the associated residence time of the gas in the gas channel until contact with the liquid to be treated, as well as the elapsing time until application in microbiological experiments, it can be assumed that the short-lived species with half-lives of at most a few seconds (Zhou et al., 2020) have no influence on the characteristics and efficacy of the PTLs, unless they are formed later on by consecutive reactions of the long-lived species. In the following discussion, we therefore mainly focus on the long-lived reactive species.

The pH value indicates the formation of strong acids in the liquid as a

result of plasma treatment. Nitrogen compounds (nitrite, nitrate, their corresponding acids and reaction products) and hydrogen peroxide are presumed to be the main reactive species responsible for the acidification of the liquid (Thirumdas et al., 2018; Zhou et al., 2018; Pan et al., 2017). However, it is known from literature that the pH does not reflect the exact concentrations of the species mentioned, especially in comparing PTLs from different base fluids which show the same pH after plasma treatment. An example of this is the study by Tsoukou et al. (2018), which showed that, despite similar pH values, the concentration of H_2O_2 and nitrate was higher in PTL from saline than in PTL from distilled water. In addition, Jyung et al. (2024) found in their study, that plasma-treated distilled water and plasma-treated NaCl solution (3.5 %) have similar pH and ORP values, but the concentrations of H_2O_2 , NO_2 , and NO_3 differed greatly. However, since in our study the pH of the S-PTL is slightly lower than the pH of the W-PTL, this could be an indicator that more strong acids were formed in the S-PTL. This assumption would be consistent with the observations of other research groups. Sampaio et al. (2022) showed in their research with PTLs produced from saline (0.9 % NaCl) and deionized water that the concentrations of H_2O_2 , HNO_2 , NO_2 , and NO_3 were higher in the PTL with lower pH. Another research group found in a comparison of plasma-treated deionized water and plasma-treated saline (0.9 % NaCl) that the pH of the plasma-treated saline was 0.1 lower than that of the plasma-treated deionized water. Interestingly, the concentrations of NO_2 , NO_3 , and HNO_3 in both PTLs were similar, but the concentration of HNO_2 in the PTL from saline was more than two fold higher than the HNO_2 concentration in the PTL from deionized water (Miranda et al., 2025).

The ORP of a liquid can be used to determine the total amount of oxidative species. It is assumed that ROS mainly influence the ORP, but individual RNS such as peroxyxynitrite and (peroxy)nitrate can also contribute to increased ORP (Wong et al., 2023; Rathore et al., 2021). Due to the fact that hydrogen peroxide can act both oxidizing and reducing, it is claimed to have the greatest influence on ORP (Tsoukou et al., 2018; Zhao et al., 2020; Wong et al., 2023). The ORP is described in the literature as a suitable parameter for evaluating the antimicrobial efficacy of fresh PTL, whereby it is assumed that the higher the ORP, the greater the antimicrobial effect (Thirumdas et al., 2018; Ma et al., 2015). We were able to confirm this assumption in our latest study investigating freshly produced S-PTL (Steinhäuser et al., 2025), therefore we are checking in our current study whether this assumption could also be stated for stored S-PTL (see Sections 3.2 and 3.3)

The electrical conductivity of deionized water with 0.003 mS/cm and of saline with 14.66 mS/cm changes due to plasma treatment to 1.017 mS/cm and 16.93 mS/cm, respectively (Fig. 3).

The increase in electrical conductivity reflects that charged particles and free ions have been introduced into the PTLs (Thirumdas et al., 2018; Wong et al., 2023; Ma et al., 2015), whereby the introduction of NO_x species during the production of PTL by arc plasma is believed to be primarily responsible for the increase in electrical conductivity (Thirumdas et al., 2018; Zhao et al., 2020). The electrical conductivity of saline before plasma treatment was higher than the electrical conductivity of deionized water due to the dissolved salts. Therefore, only the

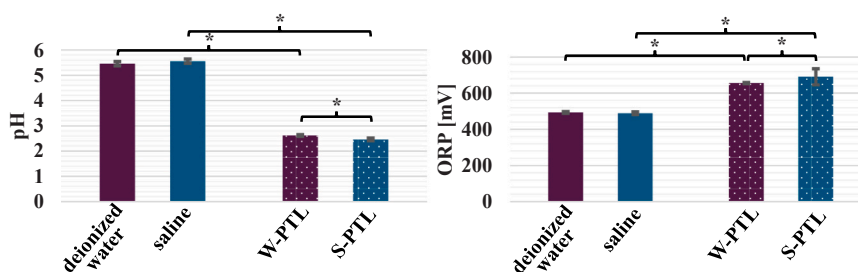


Fig. 2. Characteristics (pH and oxidation-reduction-potential (ORP)) of deionized water and saline before and after 20 min of plasma treatment (W-PTL and S-PTL). A statistical significance test was not performed between W-PTL and saline, or between S-PTL and deionized water.

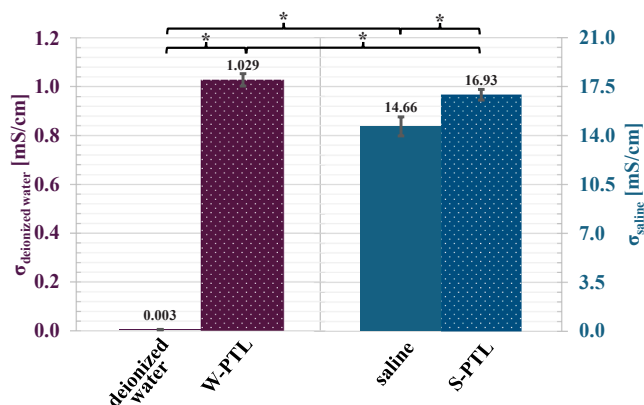


Fig. 3. Electrical conductivity (σ) of deionized water and saline before and after 20 min of plasma treatment (W-PTL and S-PTL). A statistical significance test was not performed between W-PTL and saline, or between S-PTL and deionized water.

difference between the initial value and the value after plasma treatment can be used for comparison of both liquids, whereby the increase in electrical conductivity of W-PTL with approx. 1.0 mS/cm was only half as high as the increase of S-PTL with approx. 2.2 mS/cm. The reason is still unclear, but due to the slightly lower pH and slightly higher ORP of S-PTL compared to W-PTL, it can be concluded that more reactive species, charged particles, and free ions are present in S-PTL.

3.2. Shelf life characteristics of PTL

For shelf life experiments, we used untreated saline and S-PTL, as well as untreated deionized water and W-PTL. We monitored saline (= control saline) and deionized water (= control water), as well as both PTLs either *with* regular gas exchange or *without* gas exchange during storage time of up to 21 d (Fig. 4). For the S-PTL *with* gas exchange, the monitored values remain constant in the first few minutes directly after production, i.e. the pH value is 2.5, the ORP is 690 mV and the electrical conductivity is 17.0 mS/cm (Fig. 4 and Fig. 6). The same applies to the pH and ORP of W-PTL *with* gas exchange, with values of 2.5 and 660 mV. However, the electrical conductivity of W-PTL rises sharply during the first minutes after production; over the following 60 min the rate of increase flattens and after 60 min the electrical conductivity plateaus at around 1.4 mS/cm (Fig. 4). During the next four days of the storage

experiment for S-PTL *with* gas exchange, however, a further increase in electrical conductivity to 19.3 mS/cm and an increase in ORP to 701.9 mV, as well as a further drop in pH to 1.9 is observed (Fig. 4). After 4 days, these values remain stable with slight fluctuations of ± 0.1 (pH), ± 1.4 mV (ORP) and ± 0.03 mS/cm (electrical conductivity). The sum parameters of W-PTL *with* gas exchange in the first days of storage also showed an increase in the values to 2.25 ± 0.05 for pH, 676 ± 3.1 mV for the ORP, and 2.83 ± 0.01 mS/cm for the electrical conductivity. However, stabilization of the values relative to S-PTL occurred later, on day 6 of storage.

The S-PTL *without* gas exchange also shows changes after the first 3 days of storage, but these are less prominent with values of 2.2 for pH, 684.2 mV for ORP and 17.7 mS/cm for electrical conductivity (Fig. 6). In addition, no further changes are observed at the following time points for measurement (Fig. 4, 8 d and 21 d). Interestingly, changes in pH, ORP and electrical conductivity of the stored S-PTL *without* gas exchange are significantly different between day 0 (directly after production) and day 3 of storage, whereas between day 3 and 8 this is not the case (Fig. 6). In comparison with the W-PTL *without* gas exchange, we observed a similar behavior. The values for pH, ORP and electrical conductivity changed also in the first 3 days of storage, but the changes were also less prominent with values of 2.4 (pH), 670 mV (ORP) and 1.7 mS/cm (electrical conductivity). Furthermore, no considerable changes were observed in the subsequent measurements on days 8 and 21 (Fig. 4).

The controls showed constant values: pH = 5.6 ± 0.1 , ORP = 494.6 ± 8.1 mV and $\sigma = 15.55 \pm 0.07$ mS/cm for untreated saline and pH = 5.6 ± 0.2 , ORP = 480.1 ± 10.3 mV and $\sigma = 0.009 \pm 0.004$ mS/cm for untreated deionized water over the complete period of the shelf life experiments (Fig. 4).

Our investigation on PTL storage shows that PTLs from both base liquids appear to undergo the same changes in pH, ORP and electrical conductivity as directly during plasma treatment, even when PTL production is finished (Fig. 4). Such behavior without further introduction of new reactive species by plasma gas suggests that further reactions occur in the liquid after PTL production. It has been observed in several independent studies that nitrite continues to react to nitrate after plasma treatment, i.e. the concentration of nitrite ions decreases and that of nitrate ions increases (Lukes et al., 2014; Zhao et al., 2020; Zhao et al., 2021; Zhou et al., 2020). According to some possible reaction pathways, hydrogen peroxide and ozone can also be involved in this transformation (Lukes et al., 2014; Thirumdas et al., 2018; Zhou et al., 2020). However, nitrite and hydrogen peroxide can form peroxyxynitrite in an

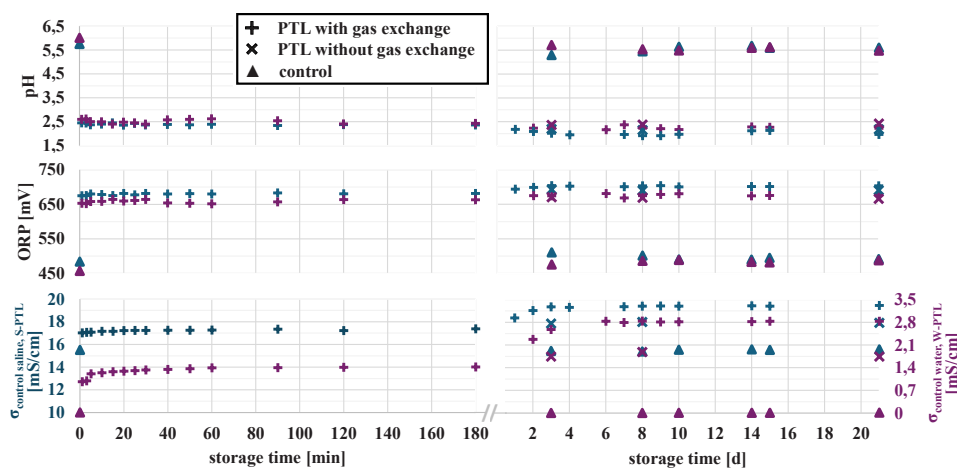


Fig. 4. Shelf life experiment for plasma-treated liquid from saline (S-PTL) and deionized water (W-PTL): monitoring of pH, ORP and electrical conductivity (σ). S-PTL (+, blue) and W-PTL (+, purple) *with* continuous gas exchange, S-PTL (x, blue) and W-PTL (x, purple) *without* gas exchange, as well as untreated saline (▲, blue) and untreated deionized water (▲, purple) as control were examined. The data shown are an exemplary excerpt. Please note that two different axes have been added for electrical conductivity.

acidic environment via different reaction pathways, which has been shown to contribute to the antimicrobial effect of PTLs (Lukes et al., 2014; Zhao et al., 2021). In contrast, Shen et al. (2016) reported that during the first days of PTL storage, the pH acidified and stagnated after approx. 15 days, but the ORP decreased during storage. Furthermore, it was observed that the concentrations of hydrogen peroxide and nitrate ions decreased steadily during storage and the concentration of nitrite ions first remained stable for a few days and afterwards also decreased (Shen et al., 2016). Wang and Salvi (2021) found in their shelf life experiments of PTL, that pH and ORP remained almost constant over a 2 d storage period, whereas electrical conductivity increased slightly. The concentration of nitrate increased in their experiments over the first few hours and then decreased again continuously, whereas the concentration of nitrite ions decreased steadily (Wang and Salvi, 2021). Compared with our previous study on S-PTLs produced with a dielectric discharge plasma source, we observed in our current study (using an arc plasma source) the opposite behavior of S-PTL during storage. In the previous study all types of S-PTL showed constant or decreasing values (pH, ORP and electrical conductivity) during storage, but never an increase of these values (Steinhäuser et al., 2025). This shows that the observations on the further reactions after the end of the plasma treatment are not consistent due to the large number of species when using different plasma sources for treatment. Therefore, we agree with literature (Tsoukou et al., 2018; Jyung et al., 2024) that without measuring the concentration of individual reactive species, no conclusions can be drawn about the influence of these individual species or reaction pathways on the values of pH, ORP and electrical conductivity.

However, as described above, nitrite, nitrate and their corresponding acids are the main contributors to the acidified pH (Rathore et al., 2021; Zhao et al., 2020; Zhao et al., 2021). It has also been reported that the conversion of nitrite to nitrate further acidifies the liquid (Zhao et al., 2020; Zhao et al., 2021). Furthermore, Rathore et al. (2021) observed that there is no direct correlation between ORP and the concentration of ozone and nitrite ions, but that the ORP also increases as the concentration of nitrate ions rises. It therefore can be supposed for the changes of the pH and ORP in our study, that the conversion of nitrite to nitrate can be assumed to be the main reason for the observed changes, independent of gas exchange. In the case of stored S-PTL *without* gas exchange, this statement can also be supported by the fact that the change of pH, ORP and electrical conductivity between day 0 and 3 of storage was significantly different (Fig. 6), indicating a conversion from nitrite to nitrate during this period. Between day 3 and 8, the values are not significantly different, which could mean that the conversion of nitrite to nitrate was completed or at least reduced to a neglectable amount after day 3.

Regarding the differences between both PTLs *with* and *without* gas exchange, it could be assumed that the gas exchange enhances the availability of nitrogen and also oxygen from the air for reactions with the reactive species in the PTL, so that further reactions can take place when gas exchange is allowed and the changes in the PTL *with* gas exchange hence are more prominent and take place for a longer time when compared to the respective PTL *without* gas exchange.

In our previous study, based on the observations of Rathore et al. (2021) we assumed that the antibacterial efficacy of PTL could be maintained due to its long-term stability, which was mainly attributed to a stable ORP value (Steinhäuser et al., 2025). Since the stored S-PTL in our current study showed higher changes in pH, ORP and electrical conductivity compared to W-PTL, as well as no decrease in ORP during storage, we focused on S-PTL for the evaluation of the antimicrobial efficacy of stored PTL (see Section 3.3).

3.3. Antibacterial efficacy of PTL

In our previous study (Steinhäuser et al., 2025), we were able to demonstrate the dependence of the antibacterial efficacy of fresh produced S-PTL on the surface material to be treated. Furthermore, we

showed that the antibacterial efficacy increased in fresh S-PTL with increased plasma treatment times, as the ORP increased. The S-PTL with the highest ORP completely reduced the bacterial inoculum independently from the surface material. Based on these results, we continued our studies on these surface materials and investigated how the antibacterial efficacy of fresh PTL is affected by a change of the base fluid and how the antibacterial efficacy of S-PTL changes during storage.

The microbiological experiments with fresh produced PTLs show that both types of PTL reduce the complete inoculum, regardless of the surface material (Fig. 5). The results with stored S-PTL show that the antibacterial efficacy decreases with increasing storage time of the S-PTL.

After 3 days of storage, the highest antibacterial effect with a complete reduction (4.9 log) is achieved on ABS, in contrast, on PVC and stainless steel a slightly smaller antibacterial efficacy is observed with a reduction of only 4.1 log and 3.9 log, respectively. Using S-PTL-8d, a further decrease in antibacterial efficacy is observed. However, the S-PTL-8d still achieves a microbial reduction of 3.7–4.1 log, depending on the surface material. It is also interesting to note that the reduction on the polymer surfaces by S-PTL-3d is higher than on stainless steel, while the opposite trend can be observed for S-PTL-8d.

In summary, a log reduction of up to 4.9 is possible. Hence, no disinfection was observed (assuming at least a 5 log (99.999 %) reduction (DIN EN 13727)). However, based on the current data, with adaptation of the experimental setup (e.g. higher bacterial inoculum) a reduction of more than 5 log also appears to be realistic, meaning that the PTL would meet the requirements for disinfection. In one of our independent data sets in this study, we even achieved this limit for disinfection with a reduction of over 5 log.

When comparing fresh S-PTL and fresh W-PTL, no differences in antibacterial efficacy were observed, which does not mean the efficacy is similar, as in both cases all bacteria were inactivated by PTL. Therefore, the experimental setup has to be adapted so that bacteria still survive after treatment of the surfaces with PTL in order to subsequently reveal any differences in efficacy. It was already shown in studies by other research groups (Sampaio et al., 2022; Jyung et al., 2024; Tsoukou et al., 2018) that there are differences between these two types of PTL and found conflicting results regarding their efficacy: For example, Jyung et al. (2024) showed in their study that PTL from saline had approximately twice the antibacterial efficacy of PTL from deionized water on *E. coli*, *S. Thyphimurium* and *L. monocytogenes*. The opposite was observed in the study by Sampaio et al. (2022), who found a multiple higher efficacy of PTL from deionized water compared to PTL from saline at various pH values in studies on *E. coli*.

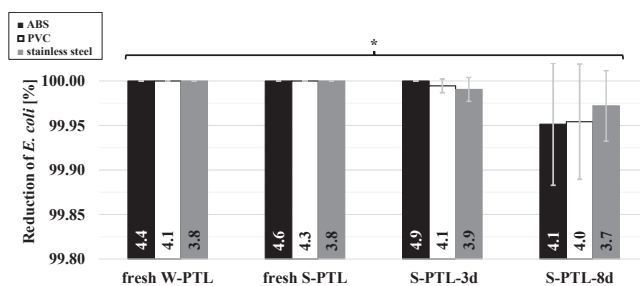


Fig. 5. Antibacterial efficacy of PTL against *E. coli* on varying substrate material: ABS (black), PVC (white) and stainless steel (grey) with variation of the base liquid used for PTL production. The numbers in the bars represent the respective log reductions. Please note that these values vary for the different materials even if 100% reduction was achieved. This is due to the different drying losses which are material dependent (see also Section 2.4.2). All data are statistically significant ($p < 0.05$) to their respective blank. No statistically significant differences could be identified between the fresh and stored PTLs on the respective materials. One of the independent data sets for fresh S-PTL are referenced from our previous study (Steinhäuser et al., 2025).

Regarding the potential formation of free or active chlorine, which could contribute to the antibacterial efficacy of S-PTL, we still assume that either free or active chlorine is not formed or is rendered ineffective by reaction with RNS. A detailed discussion of this can be found in our previous study (Steinhäuser et al., 2025). We therefore assume that chlorine species do not contribute to the antibacterial efficacy of our S-PTL.

The efficacy of S-PTL deteriorates during storage. It can therefore still be assumed that the reduced antibacterial efficacy of stored S-PTL is caused by changes in the characteristics of the PTL over the first days of storage (Fig. 4, Fig. 6). If this is the case, the antibacterial efficacy of S-PTL-3d and S-PTL-8d should be equal, since there are no further changes in characteristics after 3 days of storage (Fig. 6). This suggests that the initial increase in pH and ORP during storage correlates with a beginning decrease in antibacterial efficacy, whereas the further decrease in antibacterial efficacy between 3 d and 8 d does not correlate with stable pH, electrical conductivity, and ORP. Hence, the antibacterial efficacy of stored S-PTL cannot be monitored directly via measurement of pH, electrical conductivity and ORP and does not correlate with these physical characteristics. This is also confirmed by the fact that the changes in the characteristics of S-PTL during storage between 0 d and 3 d are significantly different (Fig. 6), but this is not reflected in a significant difference in antibacterial efficacy (Fig. 5). Thus, we cannot further confirm our previous assumption, that the antibacterial efficacy of stored PTL can be directly correlated to the ORP. In contrast, the antibacterial efficacy seems to be dependent on distinct reactive species in the PTL. We assume that these species undergo further reactions during PTL storage as stated in Section 3.2, hence their amount is decreasing which leads to a reduced antibacterial efficacy. However, these further reactions might generate other species that still contribute to the ORP and keep it on a constant level.

Our previous assumption regarding the connection of ORP and antimicrobial efficacy is based on studies of Rathore et al. (2021) on planktonic bacteria. They showed in shelf life experiments of PTL from ultrapure water for 4 weeks tested on suspensions of *Staphylococcus aureus* and *Pseudomonas aeruginosa* that if the ORP is “sufficiently high” (over 750 mV) after production, the antibacterial efficacy is also maintained. In studies on *E. coli*, Wang and Salvi (2021) in contrast found, that the antibacterial efficacy also decreases with storage of the PTL, although pH and ORP remained stable during the storage period and the fresh PTL initially allowed a complete reduction, which is in accordance with our current findings. They observed that the antibacterial activity decreased with decreasing NO_2^- concentration. Hence, they concluded that, like nitrite, other short-lived species responsible for antimicrobial efficacy dissipate and thus a reduction of the antimicrobial effect takes place. These findings are consistent with our explanations in Section 3.2

regarding the change in the characteristics of S-PTL during the first few days of storage, as we suspect that the conversion of nitrite to nitrate is the cause. The long-lived reactive species of PTL with half-lives of minutes to years include nitrates (years), nitrites (days), hydrogen peroxide (hours) and ozone (minutes) (Zhou et al., 2020). Studies on the antibacterial efficacy of the long-lived species in freshly prepared liquids against *E. coli* showed the following order of antibacterial efficacy: hydrogen peroxide > nitrite > nitrate (Zhou et al., 2018). In contrast, it has been shown that PTL containing H_2O_2 and NO_3^- exhibits lower efficacy than a PTL containing NO_2^- and NO_3^- . The higher efficacy of NO_2^- compared to H_2O_2 is attributed to nitrous acid, which is the dominant form of nitrite under acidic conditions, acting as an oxidant in an acidic environment and converting into nitrogen oxides such as NO and NO_2 , which can cause cellular damage and cell death (Fina et al., 2024), hence contributing to the antimicrobial efficacy.

The reduction in the efficacy of our S-PTL during storage can be attributed to the dissipation of most of the ROS based on the half-lives of the individual reactive species. As long as nitrite and hydrogen peroxide are present, peroxyxynitrite can be formed in an acidic environment, which has been shown to exhibit strong antibacterial activity (Lukes et al., 2014; Zhao et al., 2021; Zhou et al., 2018). Because hydrogen peroxide typically has a half-life of only a few hours, it can be expected that after several days of PTL storage, peroxyxynitrite formation is no longer supported, resulting in a reduction of antibacterial efficacy. However, based on the observations of Fina et al. (2024), we assume that nitrite, nitrous acid, NO and/or NO_2 are still present in our stored PTL and that the antibacterial efficacy is therefore still abundant.

As in our previous study (Steinhäuser et al., 2025) stated, the ORP is suited to estimate the antibacterial efficacy of fresh produced PTL and as our current results show this seems to be independent of the base liquid. But as our results (Fig. 5 and Fig. 6) also show that the ORP does not allow an estimate of the efficacy of stored S-PTL and the same accounts for pH and electrical conductivity. Therefore, other parameters and especially individual reactive species need to be measured to physically assess the antibacterial efficacy of PTL during storage.

It is important to mention that previous studies have already shown that the effectiveness of PTL on planktonic bacteria is usually higher than on adherent bacteria (Kamgang-Youbi et al., 2009). It can therefore be assumed that an increased antimicrobial efficacy can be expected when using the PTL from our study for the hygienization of liquids.

For the application of PTLs in the food industry, it will be important to additionally consider its efficacy against biofilms. Our experimental setup differs from a biofilm setup: In a biofilm, the bacteria are additionally protected by the extracellular polymeric matrix and other components of the biofilm, which would result in a comparatively reduced efficacy compared to its efficacy against adhered or planktonic

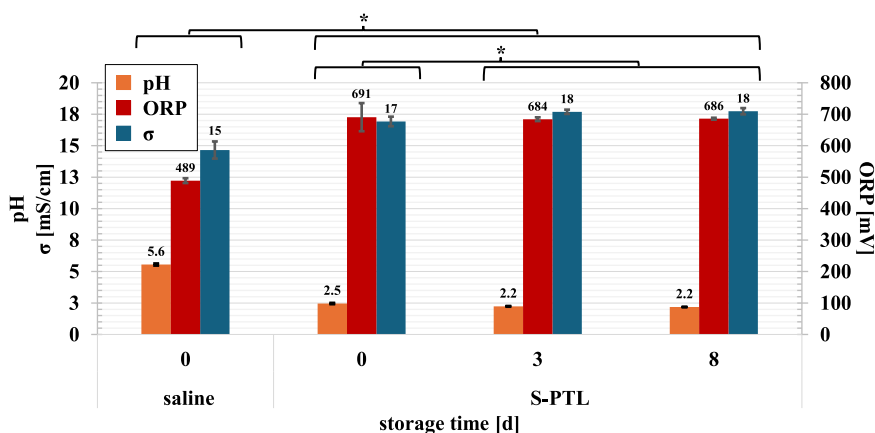


Fig. 6. Characteristics (pH, ORP and σ) of the untreated saline solution, fresh and stored S-PTL (without gas exchange) at the time of microbiological investigation. A part of the data is an excerpt from Fig. 4. All statistically significant differences refer to all depicted parameters and are marked with * ($p < 0.05$).

bacteria (Zhao et al., 2020; Kamgang-Youbi et al., 2008; Wong et al., 2023; Wang and Salvi, 2021; Smet et al., 2019). But it has already been demonstrated that PTLs are effective against biofilms (Wang and Salvi, 2021; Zhou et al., 2020; Smet et al., 2019; Xu et al., 2020). For example, in the study by Xu et al. (2020), it was shown that PTLs applied against biofilms reduce the number of bacteria, their metabolic activity, the biomass, and the membrane integrity, and markedly reduce the regrowth of the biofilm (Xu et al., 2020). Therefore, PTLs may also contribute to the control of biofilms. When PTLs are applied early in the stages of biofilm formation, they should be most effective for biofilm control, since, as shown in our study, they inactivate adherent bacteria and thereby counteract the development of a biofilm during the initial stages of biofilm formation.

Besides the differing efficacy on adherent bacteria, planktonic bacteria and biofilms, the material to be treated with PTL also plays a crucial role regarding the antimicrobial efficacy (Kamgang-Youbi et al., 2009). There are several studies on the effectiveness of PTL on surfaces (Kamgang-Youbi et al., 2008; Kamgang-Youbi et al., 2009; Kamgang-Youbi et al., 2018; Shah et al., 2024; An et al., 2019; Khan et al., 2016; Kim et al., 2018; Tan and Karwe, 2021), mainly investigating the effect of PTL on biofilms on stainless steel coupons. Only some of these studies compare different surfaces with each other. The research group Kamgang-Youbi et al. (2009) showed in their study on *S. cerevisiae* and *H. alvei* that they achieved significantly different inactivation rates by PTL treatment on stainless steel and HDPE, with a higher inactivation on HDPE. These findings are consistent with our results for S-PTL-3d, which show that PTL generally has a higher antimicrobial efficacy on polymer surfaces than on stainless steel. This leads to the assumption that there seems to be a combined influence of surface material and PTL storage time on the antimicrobial efficacy.

With regard to the differences between the used surface materials, it can be assumed that some reactive species, (e.g. those primarily responsible for the antimicrobial efficacy), react with the surface material which results in different antimicrobial effects on these materials. To substantiate this assumption and identify precise mechanisms, it is also necessary to determine and measure individual reactive species.

The observation that PTLs show different behavior on different materials suggests that this could cause damage to the material itself. There are already first indications in literature that PTL treatment is less harmful to surfaces than chemicals and does not damage them. One study has already shown that treating the surfaces of stainless steel and polyethylene with PTL had no effect on the topography of the surfaces of both materials, but that treating the surface of polyethylene with bleach solution resulted in a reduction in surface roughness and irregularities became visible under the atomic force microscope (Kamgang-Youbi et al., 2018). Another study showed that PTL is non-corrosive to stainless steel and basic non-corrosive to copper surfaces (Pan et al., 2017).

In the food industry, stainless steel is mainly used for food-contact surfaces, but a variety of different polymers are also used (e.g. for seals or valves). It is therefore important to show the influence of different materials on the effectiveness of PTL, which is why we investigated stainless steel and the two polymers PVC and ABS in our study. Furthermore, knowledge about the shelf life behavior is important for the use of PTL in industry, which we also considered. Nevertheless, our results show that detailed investigations are necessary. Further studies will therefore focus on the relationship between the individual species and the observed antimicrobial effects on the surface materials used.

4. Conclusion

Both the effect of the treated surface material as well as the storage characteristics of PTL are important parameters for an industrial application for hygienization, especially in food industry. We were able to demonstrate that fresh S-PTL and fresh W-PTL, as well as S-PTL stored for several days can reduce the bacterial contamination on surfaces up to 4.9 log, depending on the surface material. The surface material itself

has also an influence on the efficacy of PTL treatment. S-PTL has a good antibacterial efficacy of up to 4.1 log reduction after a storage time of up to 8 d. Shelf life experiments indicate that the storage conditions (e.g. gas exchange) have an influence on the stability of PTL characteristics. This point still needs to be specified in subsequent studies.

Though, we showed an antibacterial effect even after 8 d of S-PTL storage, we also observed that the antibacterial efficacy decreases during storage. As a conclusion we additionally found that there is no direct correlation between the indirect parameters pH, ORP and electrical conductivity and the antibacterial efficacy of stored S-PTL. Therefore, detailed investigations are necessary to identify measurement parameters (e.g. distinct reactive species) that can reflect the characteristics of PTL and the correlating antibacterial efficacy during storage.

CRedit authorship contribution statement

Gaby Gotzmann: Writing – review & editing, Validation, Supervision, Project administration, Conceptualization. **Linda Steinhäuser:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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