

Article

Is Industrial-Scale Wastewater Treatment Possible with a Commercially Available Atmospheric Pressure Plasma System? A Practical Study Using the Example of a Car Wash

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Abstract: The topic of water reuse is becoming increasingly important. It might be possible to use the well-known antibacterial effect of atmospheric pressure plasma due to its special mixture of reactive species, UV, and electromagnetic fields in a scaled-up, industrially interesting area to remove bacteria from wastewater, and thus, make it usable again. To review this question, water volumes of 5 L and of different qualities (turbidity and different degrees of hardness) were treated with a commercially available plasma system. The change in water-specific values such as pH, EC, ORP, nitrate, and nitrite content was determined. To test the antibacterial effect, both direct and indirect treatment of the test germ *Pseudomonas aeruginosa* was conducted. In the first case, the inoculated water samples were plasma-treated, while in the second case, the water samples were treated before inoculation with the germ. The viable bacteria were counted via the spread plate method. The best reduction rate of at least 6 log levels was achieved when inoculated deionized water samples were treated directly with plasma. A significant reduction in viability was also observed in directly treated clear tap water samples, whereby the different degrees of hardness did not influence the effectiveness of the plasma. The bacterial load remained almost unchanged when reused water samples from a car wash were treated. Based on the results, a possible application in a car wash was discussed including a cost estimation and possible limitations.

Keywords: atmospheric pressure plasma; reused water; wastewater treatment; antibacterial effect; reactive species; *Pseudomonas aeruginosa*; compressed air; industrial application



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1. Introduction

In order to prevent the scenario predicted by the WHO for the year 2030, in which several billion people will suffer from water scarcity, solutions in the field of water management should be pushed forward as soon as possible [1]. With the growing demand for water in private households, industry, and agriculture, the topic of water reuse in particular is becoming increasingly important [2]. In order to make wastewater usable again without health consequences, microbial, chemical, and pharmaceutical hazards contained therein must be removed [3]. The toxic load is determined by the process that generates the wastewater and the intended use of the recycled water determines in turn which hazard is to be removed. In applications where reused water is sprayed, special attention must be paid to the removal of microbial contaminants such as *Pseudomonas aeruginosa*, a germ that

poses a high risk for airborne transmission. For non-immunosuppressed, healthy patients, contact with *Pseudomonas aeruginosa* via the medium water mainly leads to infections of the skin and ears. However, an infection with *Pseudomonas aeruginosa* can cause pneumonia or septicemia for immunosuppressed patients, and thus, become life-threatening [4]. It is particularly feared as a nosocomial germ (i.e., germ acquired in hospital) and is also listed on the WHO priority list for antibiotic-resistant bacteria [5]. *Pseudomonas aeruginosa* is ubiquitous in the environment and colonizes engineered water systems such as drinking water distribution systems, rainwater tanks, and treated water systems, making water recycling difficult [6–8].

Several methods to reduce the microbiological loads in water are known. First and foremost, disinfection with chlorine is one of the most common industrially established processes [9]. Due to its atomic structure, chlorine offers an extraordinary oxidation potential, which is used especially for the destruction of microorganisms [10]. However, the use of chlorine is becoming more strictly regulated. One of the main reasons for this is the formation of so-called disinfection by-products (DBPs), which are hazardous to health [10]. Another notable disadvantage of chlorine use is the development of so-called chlorine-resistant bacteria (CRB). As Luo et al. and many others reported in their works, *Pseudomonas* is also one of the CRBs [9,11–13]. Another common disinfection method is UV light [14–16]. However, there are factors that reduce the effectiveness of this method as a stand-alone process. On the one hand, the process is considered unsustainable due to the risk of bacterial regeneration [17]. On the other hand, the transmittance of the water plays a major role in the effectiveness of this method [18]. In the case of a car wash, reused water is often turbid with macroscopic dirt particles. In such case, treatment with UV light would have to be preceded by a filtration step, which in turn would increase the financial and maintenance expenditure. Therefore, the use of UV light in this application often does not lead to the desired success. In the 1990s, research increasingly focused on the development of so-called advanced oxidation processes (AOP) for water and wastewater treatment. AOPs are mainly used for the removal of organic pollutants, but in recent years, research into the inactivation of bacteria with AOPs has grown rapidly [19–21]. Common to all AOP processes is the formation of very reactive hydroxyl radicals, which are also considered to play a key role in the use of non-thermal plasmas for sterilization and disinfection purposes [22,23]. However, one disadvantage of some common AOPs, as with the use of chlorine, is the formation of DBPs, which can pose problems with regard to future stricter regulations [24].

An exciting alternative to the much researched AOPs and other methods can be the treatment of water with atmospheric pressure plasmas due to their unique chemistry. According to numerous research studies, the reactive species generated in the ionized gas are considered to be effective in inactivating microorganisms [25–28]. Their exact composition is determined by the adjustable plasma parameters and the gas used [29]. Research results show that UV, thermal radiation, electromagnetic fields, and charged particles also contribute to the inactivation of pathogens [30–32]. In addition to the chemical and electrical mechanisms of action, water treatment by plasma (due to bubbles or pulses) also generates shock waves that can mechanically contribute to the destruction of target organisms [33]. Plasma, thus, involves several mechanisms of action simultaneously and is not limited by such factor as turbidity of the treated wastewater. In case of water treatment, non-thermal plasma sources are understood to have the highest efficiency [34]. The effectiveness of an antibacterial effect of a plasma treatment depends not only on the type of plasma source used, but also on the characteristics of the target organisms to be treated [35]. For example, Mai-Prochnow et al. showed that Gram-negative bacteria like *Pseudomonas aeruginosa* are more “plasma-sensitive” than Gram-positive bacteria [36].

Furthermore, biofilm-forming germs, to which *Pseudomonas aeruginosa* also belongs, have been reported to be more resistant against antimicrobial agents than bacteria in a planktonic state [37,38]. The inactivation of this resistant life form is a challenge, and therefore, the subject of many studies [39].

When water is treated with plasma, both long-lived and relatively short-lived chemical species, known as reactive oxygen and nitrogen species (RONS), are produced, resulting in plasma-activated water (PAW) [40]. One advantage of plasma-activated water over plasma gas in antimicrobial applications is the extension of the lifetime of certain species in water: some short-lived species produced in the plasma react with water molecules and form long-lived species [41]. This in turn proves to be an advantage in the fight against biofilms. A mixture of different long-lived reactive species can penetrate deeper into the cell structure and act longer—a synergistic effect that enables their destruction [35]. The reaction of the short-lived nitrogen species with oxygen and hydrogen-containing radicals results in increased nitrite and nitrate concentrations. A detailed description of the chemistry and the generation mechanism of nitrites and nitrates in PAW can be found in [40]. Plasma-activated water is also characterised by a reduction in the pH value [42]. The composition of the treated water sample plays a role in the effectiveness of plasma treatment. As investigated in [43], water hardness has a significant influence on the concentration of the active species in the treated sample. Therefore, the buffering effect of the water poses a particular challenge for a successful plasma treatment. In addition, other chemical compounds present in the sample can react with the active species of the plasma, and thus, reduce their concentration. Such substances are often referred to as scavengers [44].

The main objective of this work is to investigate the antibacterial effect of an atmospheric pressure plasma in case of treatment of water with special attention to the industrial feasibility of a plasma treatment process. Thus, water samples of different degrees of hardness: deionized water, tap water, and polluted water from a car wash, which also contained other chemicals and biodegradable pollutants, were tested. While several research groups have only considered small water volumes less than 50 µL to prove the antibacterial effect on *Pseudomonas aeruginosa* [45–47], much larger volumes need to be dealt with, especially when treating wastewater of industrial plants. A sample volume of 5 L is, therefore, chosen in this work. With the industrial feasibility in mind, a commercially available plasma system was chosen for the investigations. The plasma system used as well as the experimental setup and other preparatory steps are described in Section 2. In the experiments, time-dependent direct and indirect treatments were concluded. The influence of the plasma treatment time and the exposure time of bacteria to the plasma-treated water was examined. In addition, an attempt was made to assess the after-effect of the plasma-treated water, i.e., how long the plasma-treated water retained its antibacterial properties. All results presented are based on the viable count of *Pseudomonas aeruginosa* inoculated in water samples of different qualities. The results of the direct treatment are presented in Section 3, while the results of the indirect treatment in Section 4, followed by a discussion in Section 5.

2. Experimental Realization

2.1. Plasma Equipment

The plasma system used is the plasmabrush® PB3 from relyon plasma GmbH, Regensburg, Germany. The system consists of a plasma generator PG31, shown in Figure 1, and a power supply unit PS2000. For the experiments, an A450 nozzle with an outlet diameter of 4 mm is used [48]. All of the above components are manufactured by relyon plasma GmbH, Regensburg, Germany.

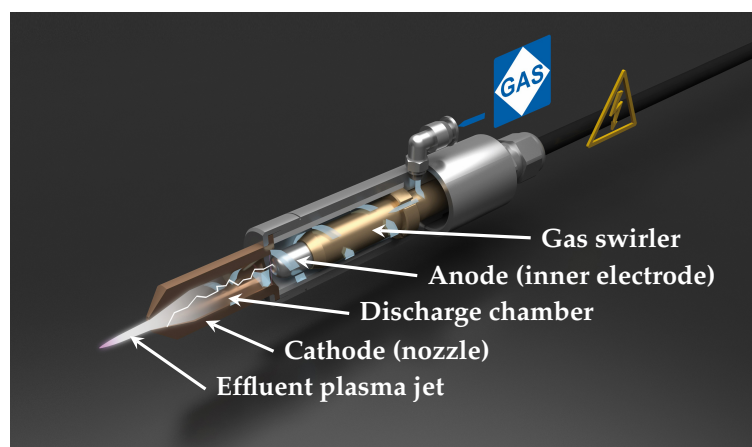


Figure 1. Schematic representation of the plasma generator PG31 used from the plasmabrush® PB3 system (relyon plasma GmbH).

The operating principle of this system is based on a pulsed low-current high-voltage discharge, whereby unipolar, triangular-shaped current pulses with a variable frequency ranging from 40 kHz to 65 kHz and variable amplitude between 0.7 A and 1.0 A are generated by the power supply. The system can be operated with different plasma carrier gases—nitrogen or compressed air are most commonly used. A detailed description of the system and the generated plasma can be found in [49,50]. For the experiments, a pulse frequency of 60 kHz and a power setting of 100 %, corresponding to a current amplitude of 1.0 A, are set as constant test parameters. Furthermore, compressed air with a volume flow of 35 L/min is used as plasma gas. From an economic perspective, compressed air offers the advantage for an end application because it is available in most industrial environments. The constant parameters set during the experiments are summarized in Table 1.

Table 1. Constant treatment parameters during all experiments.

Distance	Gas	Flow	Power	Frequency	Nozzle
20 mm	DCA	35 L/min	100%	60 kHz	A450

2.2. Experimental Setup

The experimental setup used for the plasma treatment of water samples is schematically shown in Figure 2. As mentioned in the introduction, the samples had a volume of 5 liters for each test run regardless of the treatment type (see Sections 3 and 4). The distance between the nozzle outlet of the plasma generator and the water surface was set to 20 mm. A stirrer (RZR 2102 control, Heidolph, Schwabach, Germany) at 510 rpm with cone-shaped stirring nozzles (of type VISCO JET® with a diameter of 80 mm, also made by Heidolph, Schwabach, Germany) was used for homogeneous mixing of the sample solution during plasma treatment. The reactor was closed during treatment in order to increase the concentration of reactive species, and thus, the effectiveness of the treatment [40]. On the right-hand side of Figure 2, the plasma jet is shown during treatment of the sample volume.

The effect of the plasma treatment could be influenced by an increase in water temperature due to the energy input of the plasma, especially with longer treatment times. A maximum water temperature of 53 °C was reached after 30 min of treatment, a temperature high enough to influence the microbiological results according to Spinks et al. and Tsuji et al. [51,52]. To mitigate this, a cooling system was integrated into the setup, as shown in Figure 2. The cooling system ensures that the water temperature does not exceed 40 °C after the longest treatment times tested, thus ensuring that the temperature

has no effect on the viability of the bacteria. In case of a car wash, for example, much larger quantities of water have to be treated, so that additional cooling will not be necessary.

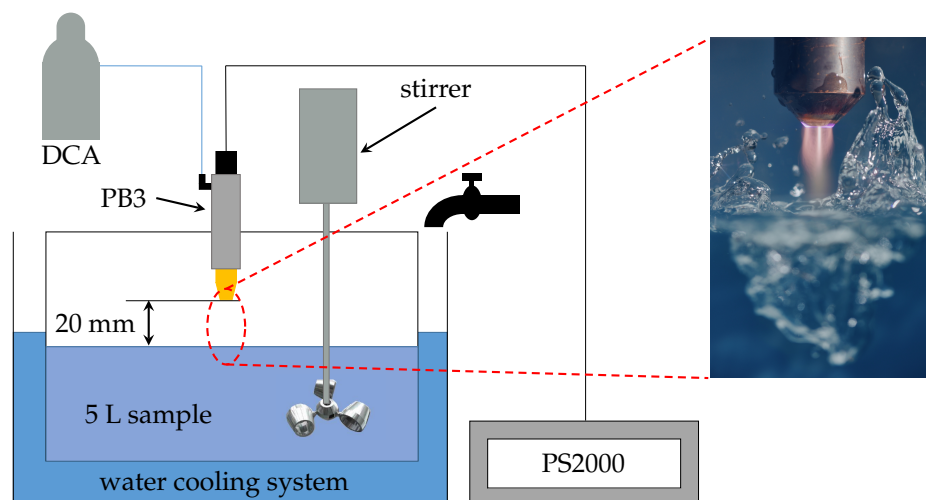


Figure 2. Schematic diagram of the experimental setup on the left side. The plasma generator was placed 20 mm above the water surface and the reactor was closed during treatment. The picture on the right side shows the generator during operation.

2.3. Preparation of Water Samples and Chemical Measurements

In order to assess the influence of water composition on the effectiveness of plasma treatment, the following water samples were examined in this work:

- Deionized water (DI);
- Tap water with two different degrees of hardness;
- Reused water from a car wash.

The samples, therefore, differ in mineral content as well as in the type of contamination and turbidity. One of the tap water samples was hardened to a predefined target value according to DIN EN 1276 [53], which suggests a standardized water hardness of 21 °dH. In contrast to the standard, real tap water was hardened here instead of DI water in order to preserve the naturally occurring components of tap water. For this purpose, two different solutions, labeled A and B, were mixed in a volume with a ratio of 3:4 and added to the sample. Solution A mixed 19.84 g/L magnesium chloride (MgCl_2) with 46.24 g/L calcium chloride (CaCl_2) in distilled water, while solution B mixed 35.02 g/L sodium bicarbonate (NaHCO_3) in distilled water. Both solutions were sterilized by membrane filtration before addition to the sample volume. A total of 1 mL of the mixed solution A increases the hardness by 3.51 °dH (resulting in 62.48 ppm CaCO_3 or 0.625 mmol/L alkaline earth ions). After mixing, the hardness of all water samples was determined using a titrimetric total hardness test of the type MColortest (Art. No. 1.11104.0001) from Merck KGaA, Darmstadt, Germany, and compared in Table 2.

Table 2. Water hardness of the analyzed water samples.

Water Type	Deionized Water	Tap Water	Hardened Tap Water
Hardness	2 °dH	13–14 °dH	21–22 °dH

The reused water samples were contaminated with various substances. According to the supplier of the reused water, the following substances are found in the samples, listed in

order of concentration from high to low: alkalis (NaOH), complexing agents (MGDA), and dispersants (phosphonates), followed by various surfactants. These samples also contained suspended particles of different sizes and sediments. A detailed chemical analysis of the reused water is not included here, as this would go beyond the scope of this paper. Due to the high chemical load, the titrimetric hardness test method was not applicable for determining the hardness of the reused samples.

The following parameters were used to assess the results of a treatment: the pH value (pH meter: pH 3110 Basic, Carl Roth, Karlsruhe, Germany), the electrical conductivity (short EC) (EC meter: Measury TDS & EC Meter, PLB Products, Hannover, Germany), and the oxidation reduction potential (short ORP) value (ORP meter: ORP-169E, Kuuleyn, Shenzhen Yilaijia Technology Co., Ltd., Shenzhen, China). The parameters were measured for both the blank and treated samples.

2.4. Cultivation of Bacteria and Preparation of Test Suspensions

Pathogenic *Pseudomonas aeruginosa* ATCC 15442 [54] was subcultured from the stock culture on TSA (Tryptic Soy Agar, Carl Roth GmbH + Co. KG, Karlsruhe, Germany, cat. no. X937 [55], with 15 g/L casein peptone, 5 g/L soy peptone, 5 g/L sodium chloride, and 15 g/L agar, pH = 7.3 ± 0.2), and incubated overnight (appr. 20 to 24 h) at 37 °C. From this overnight culture, a second subculture was prepared either on TSA and incubated overnight at 37 °C (for the indirect treatment procedure) or in TSB (Tryptic Soy Broth, Carl Roth GmbH + Co. KG, Karlsruhe, Germany, cat. no. X938: 17 g/L casein peptone, 3 g/L soy peptone, 2.5 g/L di-potassium hydrogen phosphate, 5 g/L sodium chloride, 2.5 g/L glucose monohydrate, pH = 7.3 ± 0.2) and incubated overnight at 37 °C and 200 rpm on an orbital shaker (for the direct treatment procedure).

3. Direct Treatment

The aim of this series of tests is to assess the plasma effect for a real-life scenario, as it could be used in a potential application. For this purpose, plasma was in direct contact with the inoculated water samples and a so-called direct treatment took place.

3.1. Materials and Methods

The bacterial suspensions were prepared from a liquid overnight culture. The culture was harvested by centrifugation at 10,410 g and the pellet was resuspended in an appropriate amount of dilution medium (8.5 g/L NaCl, 1 g/L tryptone from casein) in order to yield a bacterial concentration of about 10^9 CFU/mL. The water samples were then inoculated with 500 mL of the adjusted bacterial solution in order to achieve an initial count of 10^8 CFU/mL prior to the direct treatment with plasma.

To determine the die-off kinetics, samples were taken before treatment (marked as 0 min) and after 10, 20, and 30 min of plasma treatment. Before plating onto TSA plates, the aliquots of 0.5 mL of the treated suspension were suspended in 4.5 mL of neutralization medium (30 g/L polysorbate 80, 30 g/L Saponine, 3 g/L Lecithine, 1 g/L Histidine, and 5 g/L sodium thiosulfate) for 10 s to “divert” the chemical reactions and prevent an after-effect of the plasma-treated water. More specifically, the reducing agent sodium thiosulphate reacts with the oxidizing agents generated in the plasma so that they can no longer oxidize the bacterial components, thereby eliminating an after-effect. Neutralizing chemical reactions in this way is recommended when testing chemical disinfectants and antiseptics according to DIN EN 1276. The volume of the aliquots could be kept low at 0.5 mL, as the sample is permanently homogenized with the stirrer and the volume of the sample did not change significantly with each sampling. After neutralization, 0.1 mL of appropriate serial 10-fold dilutions were plated onto TSA plates and incubated at 37 °C for

48 h. Colonies were counted after 24 and 48 h of incubation and CFU values of surviving bacteria per mL were calculated. A pass level of 5 log₁₀ reduction in viable bacterial counts was defined in accordance with European Standards for efficacy testing [56].

Additionally, when analyzing the influence of impurities and turbidity, an attempt was made to estimate the influence of plasma on the naturally occurring flora on the one hand and on the target organisms on the other. As the concentration of the target organism (i.e., *Pseudomonas*) was spiked, it was more difficult for the natural flora to prevail. Nevertheless, the experiment was undertaken to check whether other organisms could play a possible role. For differentiation, a universal medium (TSA/CASO agar, see Section 2.4) and a selective medium (Cetrimide agar [57]: gelatine peptone 20 g/L, magnesium chloride 1.4 g/L, potassium sulfate 10 g/L, cetrimide 0.3 g/L, and agar 15 g/L) with a final pH of 7.2 ± 0.2 (at 25 °C) were used for cultivation.

3.2. Results

The effectiveness of plasma treatment is important when using atmospheric pressure plasma for water treatment, especially with regard to industrial applications. As described in Section 1, there are a number of factors that can contribute to this effectiveness. In the following section, the change in water-specific values is first investigated. Then, the experimental results focusing on the antibacterial effect of the chosen plasma system are presented.

3.2.1. Influence on pH Value, Nitrite Concentration, and Nitrate Concentration

Since the effectiveness of the plasma treatment depends on the buffering properties of the respective water, a relationship between initial water hardness and chemical changes in the water should first be explored with the here used plasma system and the investigated water probes [42]. First of all, water samples with three different degrees of hardness (see Table 2) and a volume of 5 L were treated with plasma for 30 min and then the pH values were measured.

The results summarized in Table 3 show, as expected on the basis of numerous research results such as [40,58–61], that in general plasma treatment reduces the pH value of water. However, the strength of the pH-reduction depends on the original water composition. For tap water with an initial hardness of 15 °dH as well as for tap water hardened to 22 °dH, a small reduction of the pH value of ~0.6 and ~1.2 could be measured, respectively. Deionized water with an initial hardness of 2 °dH, on the other hand, experiences a significant acidification, with the pH value falling from ~8.54 to ~2.99 after 30 min of plasma treatment. As can be seen in Table 3, plasma treatment generally increases the electrical conductivity of water. This is mainly due to the charged particles generated by the plasma, which are transferred into the water. The greatest impact of plasma treatment is achieved with DI water, where an increase in electrical conductivity of more than 700 µS/cm is recorded. A smaller but still significant increase in conductivity can also be observed in the other water samples. The oxidation reduction potential, on the other hand, only increases with the treated deionized sample.

As the experimental results also show, an increase in both nitrite and nitrate concentrations is observed after treatment. The nitrate concentration of DI water increases significantly from less than 1 mg/L to 122 mg/L. However, noticeably less nitrate is formed in the samples with higher degrees of hardness, namely 22 mg/L in tap water with a hardness of 15 °dH and 18.5 mg/L in tap water with 22 °dH. In contrast, a significantly lower nitrite concentration of 68 mg/L is measured in the deionized sample than in samples with higher hardness values, namely 200 mg/L for tap water or 190 mg/L for hardened tap water. A possible reason for this could be the higher pH value (see Table 3) and the buffering capacity of the plasma treated

hardened water. Other chemical elements listed in Table 3 remain almost unchanged after a treatment. The higher chloride concentration of the hardened tap water samples is due to the addition of the hardening solutions. It can, therefore, be assumed that the nitrogen-containing compounds make up the majority of the reactive species in the plasma-treated samples.

Table 3. Physicochemical properties of the different water samples, measured before and after a 30 min plasma treatment. The differences between untreated and treated values are marked in green.

Parameter	Deionized Water			Tap Water					
	Untreated	Treated	Diff	Untreated	Treated	Diff	Hardened Untreated	Hardened Treated	Diff
pH [°dH]	8.54	2.99	−5.55	6.90	6.32	−0.58	8.09	6.88	−1.21
EC [µS/cm]	50.4	758	+707.6	486	637	+151	821	989	+168
ORP [mV]	253	546	+293	367	341	−26	300	264	−36
nitrite [mg/L]	0.1	68	+67.9	0.1	200	+199.9	0.1	190	+189.9
nitrate [mg/L]	0.6	122	+121.4	3.0	22	+19	3.0	18.5	+15.5
fluoride [mg/L]	<0.1	<0.1	0.0	0.18	0.16	−0.02	0.18	0.16	−0.02
chloride [mg/L]	0.7	0.7	0.0	3.8	3.9	+0.1	98	94	−4
phosphate [mg/L]	0.4	<0.1	−0.4	<0.1	<0.1	0.0	<0.1	<0.1	0.0
sulfate [mg/L]	0.37	0.4	+0.03	10.3	10.4	+0.1	10.3	10.8	+0.5
TOC [mg/L]	0.28	0.57	+0.29	0.37	0.66	+0.29	0.69	0.78	+0.09

The absorbance of UV light is an established method to monitor water quality. It can also be used to indicate the concentration of nitrites and nitrates in a water sample [62,63]. As shown in Figure 3, all plasma-treated samples exhibited significantly increased absorption at wavelengths below 240 nm, which correlates well with the absorbance wavelengths of NO_2^- and NO_3^- [62,64] and the values given in Table 3. However, distinguishing between nitrite and nitrate concentrations (or other chemical compounds) solely on the basis of UV absorption spectra requires advanced analytical methods [63–65], which is beyond the scope of this paper.

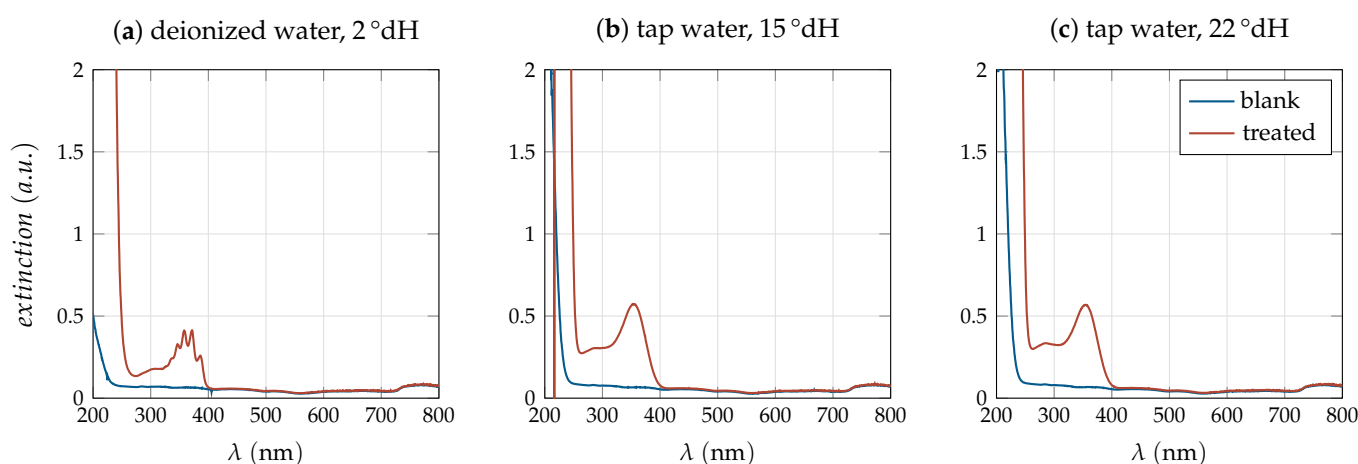


Figure 3. UV absorbance spectra. The sharp increase in the values below 240 nm indicates increased nitrite and nitrate concentrations in the treated samples.

3.2.2. Antibacterial Effects

In the next step, the antibacterial effects of plasma were assessed for samples with different water hardness. Figure 4 shows live cell counts of *Pseudomonas aeruginosa* after a direct plasma treatment (see Section 3.1) depending of the treatment time of 0, 10, 20, and

30 min. Plasma treatment of deionized water achieves the best results. After 10 min of plasma treatment, the viable cell count shows a decrease of ~ 1.5 log levels. After a 20 min treatment, the bacterial count was reduced to such an extent that it was below the detection limit of 1400 CFU/mL (lowest plated dilution level 10^{-1} and plated volume of 0.1 mL). The different degrees of hardness of the treated tap water samples has no significant influence on the viability of the cells.

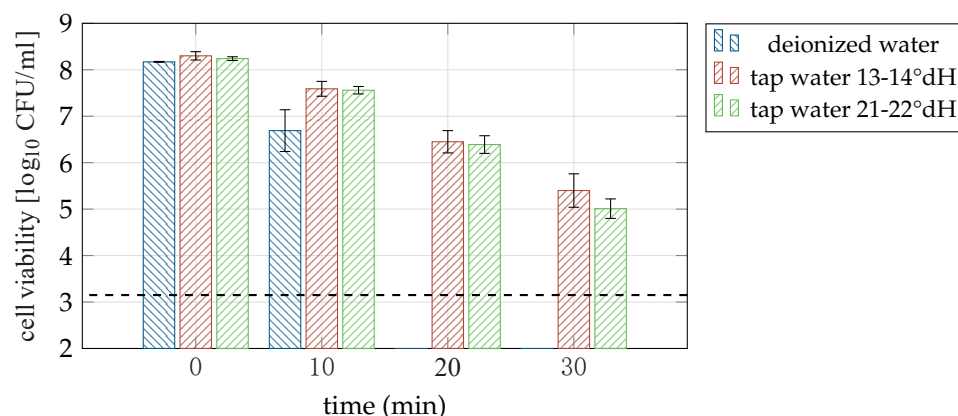


Figure 4. Viable cell counts of *Pseudomonas aeruginosa* as a function of plasma treatment time. The dashed line shows the detection limit. The experiments were conducted with water cooling (see Section 2.2) so that the sample temperature remained below 40 °C after the longest treatment time.

3.2.3. Influence of Impurities and Turbidity on the Effectiveness of Plasma Treatment

Figure 5 shows the die-off kinetics of *Pseudomonas aeruginosa* after treatment of clear tap water and polluted reused water, with the treatment time of the sample volume being limited to 30 min. After every 5 min of treatment, 0.5 mL aliquots were taken and neutralized in 4.5 mL of the neutralization medium, as described in Section 3.1, in order to subsequently determine the number of microorganisms still present. Then, the aliquots were plated onto different media, TSA and *Pseudomonas*-selective Cetrinide. As can be seen in Figure 5, a clear decrease of the bacterial load can be observed in correlation to the duration of the plasma treatment for tap water (Figure 5a) and reused water (Figure 5b). With a reduction of about 5 log levels after 25 min of treatment, the decrease in CFU/mL for tap water is significant and an approximate D value of 5 min can be determined for the parameter set used. The D value is the decimal reduction value and indicates the time required for a reduction of the bacterial concentration by 90 %, i.e., 1 log level. In contrast to tap water, the antibacterial effect of plasma is significantly lower when treating reused water. A reduction of approximately 1 log level can only be determined after a treatment time of 30 min. While a continuous decrease in the number of bacteria can be seen over time when treating tap water, this is not the case when treating reused water.

In order to test the influence on the naturally existing flora of the different water samples on the one hand and the target organisms (*Pseudomonas*) on the other hand in a non-artificially modified matrix, two different types of agar were used: TSA (Tryptic Soy Agar), which is a universal medium, and Cetrinide (Cetrinide-Agar), which is selective for *Pseudomonas*. As can be seen in Figure 5, the reduction rates for tap water or reused water behave similarly and are, therefore, considered to be independent of the selected cultivation medium.

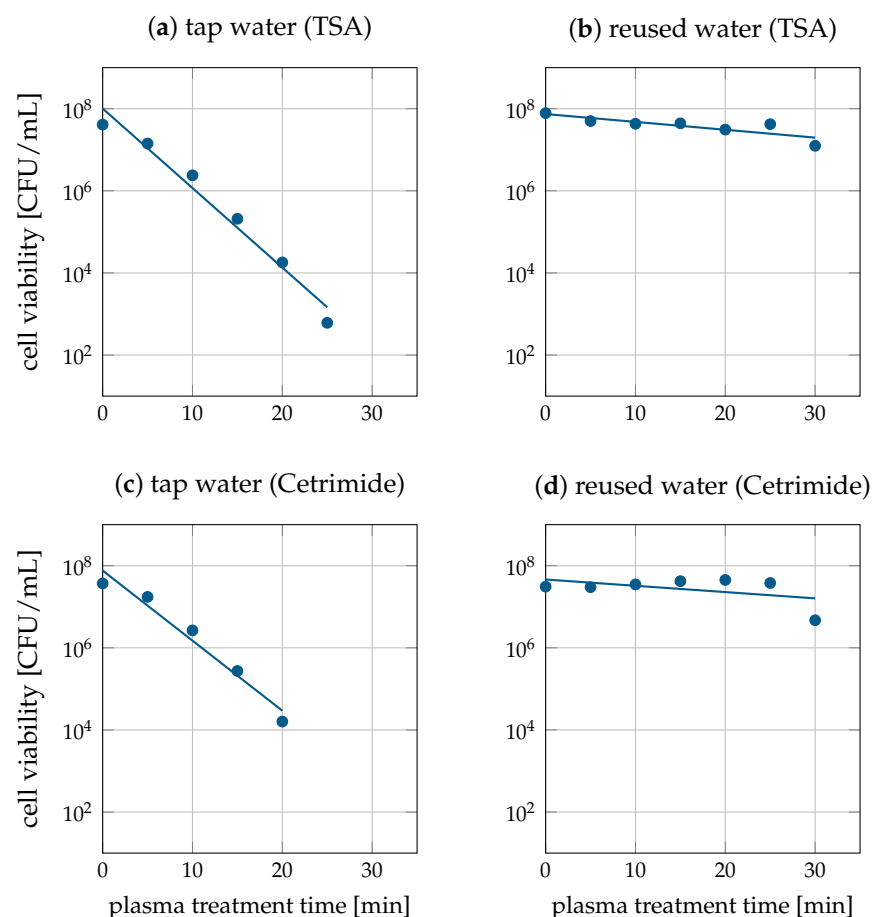


Figure 5. Die-off kinetics of *Pseudomonas aeruginosa* as a function of plasma treatment time. We treated 5 L of tap water and 5 L of reused water and we examined the number of bacteria at intervals of 5 min with a maximum treatment time of 30 min. Furthermore, two different culture media were used (TSA and *Pseudomonas*-selective Cetrimide). The experiments were conducted without sample cooling (see Section 2.2), which resulted in a sample temperature of 53 °C after the longest treatment time.

4. Indirect Treatment

Plasma-treated water is known to retain its bactericidal properties over an extended period of time, as reported in [58,66–68], to name just a few examples. Such bactericidal remanence is of interest with regard to industrial application, especially where water circulates in closed or semi-closed systems. To estimate this effect, the stability of PAW over time was first assessed. Then, different water samples with a volume of 5 L were treated with the experimental setup for 30 min. Finally, the bacteria were exposed to these water samples for different lengths of time, whereby the plasma treatment took place 3 h beforehand. In this way, the bacteria were not in direct contact with the plasma (as in Section 3), but were only brought in contact with the plasma-treated water samples. Therefore, the results observed in this section can only be attributed to the bactericidal remanence effect of the PAW.

4.1. Stability of PAW over Time

The stability of plasma-treated water over time is an important parameter, particularly when indirect treatment is considered. To estimate the long-term stability, tap water and deionized water were treated with plasma for 10 min and then pH, EC, and ORP values were measured at different time intervals up to 48 h after treatment. The results are shown in Figure 6.

The change in the physicochemical properties of deionized water after plasma treatment is significant, as already indicated in Table 3. The pH value of DI water is lowered to about 3 and remains constant over 48 h. The ORP value increases directly after treatment, but then also remains almost constant over the time analyzed. The conductivity, on the other hand, increases slowly over time, which could be an indicator of chemical reactions occurring in the deionized sample. In contrast to that, the reduced pH value of tap water after treatment returns to its initial value after two days. However, the EC and ORP values of tap water can be regarded as constant, if the ORP value after 3 h is considered an outlier.

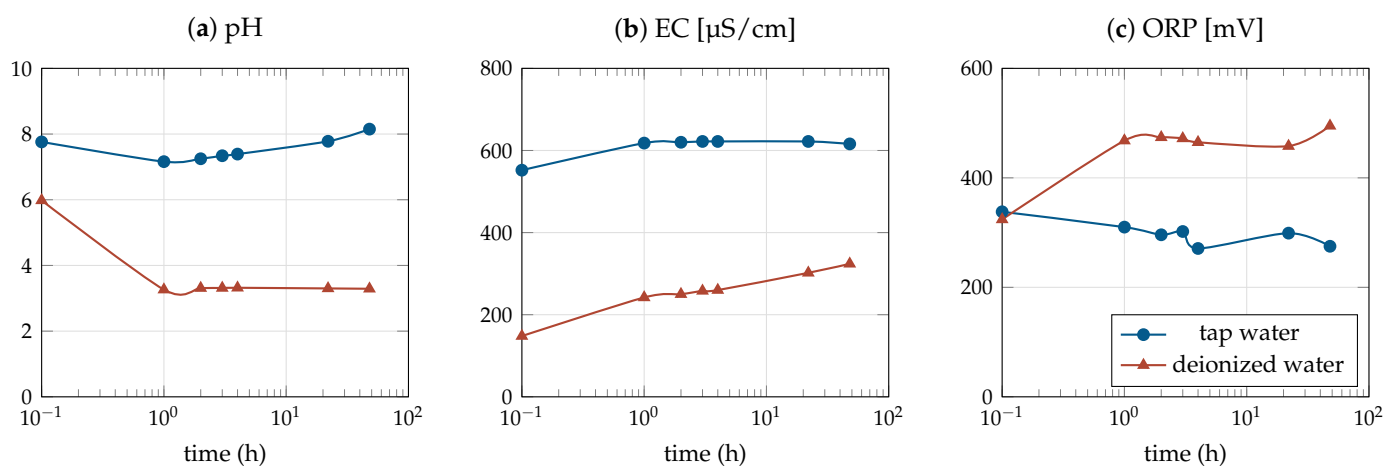


Figure 6. Stability of PAW over a period of 48 h after 10 min of plasma treatment. The first mark at 10^{-1} in each plot refers to the values measured for blank (i.e., untreated) water samples. The values of pH, EC, and ORP were measured after 1, 2, 3, 4, 22, and 48 h after plasma treatment.

Considering the above results, a working hypothesis can be put forward that DI water has a higher long-term stability than tap water due to the overall significantly greater change in physicochemical properties. Nevertheless, the bactericidal effect may decrease over time due to chemical reactions occurring within the sample. To verify this, the stability of the bactericidal effect of PAW over time was tested by cell counting according to the following method.

4.2. Materials and Methods

The bacterial suspension was prepared by harvesting colonies from TSA plates and diluting them in an appropriate amount of dilution medium to yield a density of 1.7×10^9 CFU/mL, thereby modifying the requirements of DIN EN 1276 in order to start with an initial count comparable to that in the direct treatment. This suspension was diluted 10-fold in the subsequent quantitative suspension test to the initial density of 1.17×10^8 CFU/mL (t_0). For all test series, with the exception of DI water, the samples were autoclaved in a first step.

To evaluate the bactericidal remanence effect of plasma-treated water, a quantitative suspension test was performed in accordance with DIN EN 1276: treated water samples were used as the product test solution and autoclaved reused water as the interfering substance. Briefly, 1 mL of the *Pseudomonas* test suspension was mixed with 1 mL of untreated, autoclaved reused water. After 2 min, 8 mL of the respective plasma-treated water sample was added and after exposure times of 5 or 10 min, respectively, aliquots of 1 mL were transferred to test tubes with 8 mL of neutralization medium. After a neutralization time of 10 s, 1 mL of this mixture as well as 1 mL of a 10-fold dilution of it were plated onto TSA (two plates each) and incubated at 37 °C for 48 h. Colonies were counted after 24 h and 48 h of incubation and CFU values of surviving bacteria per mL

were calculated. Similar to the evaluation methodology of the direct treatment tests (see Section 3.1), a pass level of 5 log₁₀ reduction in viable bacterial counts was defined in accordance with European Standards for efficacy testing.

4.3. Results

The results in Figure 7 show a strong effect of plasma-activated water on *Pseudomonas aeruginosa* in case of deionized water. The application of plasma-activated deionized water (applied approximately 3 h after plasma treatment), which was in contact with the bacteria for 5 min, reduced the bacterial count by 5.04 log levels. With an application time of 10 min, a reduction of 5.92 log levels was even achieved. The same tests to investigate the after-effects of PAW were also carried out for autoclaved tap water and autoclaved reused water. In these test series, however, the bacterial counts after treatment were above the evaluable range, which means a maximum reduction of 3.25 log levels. Due to that, they are not shown in Figure 7.

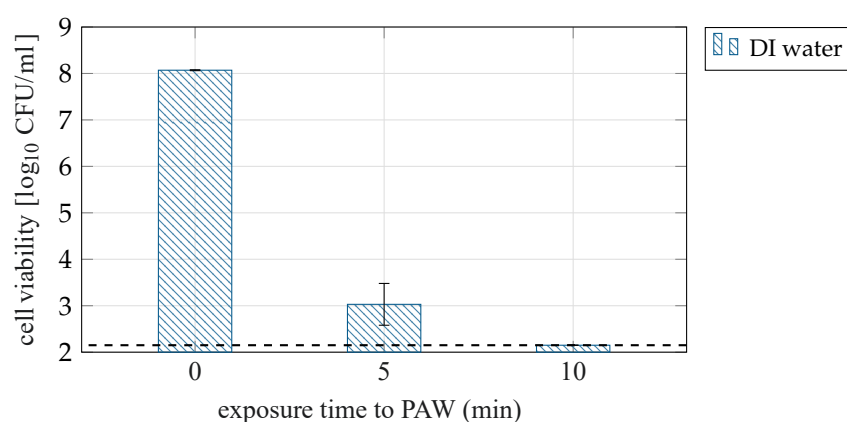


Figure 7. Viable cell counts of *Pseudomonas aeruginosa* as a function of exposure time to plasma-treated DI water. The water sample was treated for 30 min and applied approximately 3 h later. The dashed line shows the detection limit.

Although bactericidal remanence was only assessed for samples that were plasma-treated 3 h before performing the indirect treatment tests, the results seem to support the hypothesis that DI water has a higher long-term stability (i.e., it retains its antimicrobial properties longer) than other water samples. However, further studies are needed to confirm this statement, which is beyond the scope of this work.

5. Discussion

5.1. Comparison with Previous Work

The aim of this work was to investigate the antimicrobial effect of atmospheric pressure plasma when treating water in an industrial context. This assumption was decisive for the choice of the plasma system, which is an industrially proven and commercially available device that operates with a pulsed streamer-spark discharge. Such a discharge is used by most industrial plasma devices with a power in the range of around 1 kW and has been used in the past for water treatment [58,59]. According to the results shown in Figure 4, a significant reduction in cell viability was observed with deionized and clear tap water, with the highest reduction rate of at least 6 log levels achieved after 20 min of treatment with the former. In [58], Joshi et al. used a commercially available plasma system comparable to the one used here to investigate the antimicrobial efficacy of plasma-treated water and plasma-activated acidified buffer. The change in pH, EC, and ORP values reported by the authors for deionized water after a 5 min treatment agrees well with the ones observed here. Although different germs were used in their study, the reduction rate with deionized water

was also similar, reaching about 2 log levels after 10 min of treatment (see Figure 4). Tan and Karwe [59] used the same plasma system as Joshi et al. to investigate the influence on biofilms in piping systems. The authors measured similar values to those given in [58] for pH, EC, and ORP after a treatment time of 5 min for deionized water. They also reported a concentration of nitrites in the range of 40 ppm and nitrates of 250 ppm after treatment. In comparison, almost twice the concentration of NO_2^- and half the concentration of NO_3^- was measured here. The discrepancy is probably due to a different concentration of the species in the gas phase, which are then transferred into the water, or due to a much higher sample volume in this study. The different experimental setup could also play an important role here. Other authors who used comparable but not commercially available plasma generators such as gliding arcs operated at frequencies in the range of several tens of kilohertz or various high-voltage low-current discharges reported similar behavior of the physical properties of treated DI water [60,61]. Thus, despite the inevitable differences in the experimental setups as well as sample volumes, the results achieved for DI water are considered plausible and in good agreement with previous work.

5.2. Possible Inactivation Mechanisms of *Pseudomonas*

The inactivation of *Pseudomonas aeruginosa* by plasma-treated liquids has been investigated in several studies [26,61,69]. Kondeti et al., who investigated the influence of short- and long-lived species on a multidrug-resistant *Pseudomonas* strain PA14 in [26], reported that inactivation strongly depends on the chemical composition, and thus, the buffering capacity of the treated medium. In the presence of NaCl, the long-lived ClO^- is considered to be the main working agent. However, the effect of ClO^- is dominant at pH values above 7.5. This is usually not the case for water, as the plasma treatment tends to acidify the samples, as shown in [26] and in Table 3. According to the authors, slightly better results were achieved with an oxygen-containing plasma when treating water than with an air plasma. The effect is attributed to short-lived oxygen species ($\cdot\text{OH}$ and $\text{O}_2^{\cdot-}$), which are transferred into the liquid and form $\cdot\text{O}$ as well as $\text{O}_2^{\cdot-}$ [26]. Unfortunately, the authors did not analyze the inactivation mechanisms for nitrogen-containing air plasma to a similar extent. In contrast, Modic et al., who investigated the influence of plasma on biofilms of the PAO1 strain in [69], observed that the inactivation of *Pseudomonas* was much higher under RNS-dominated conditions than under ROS-dominated ones. The reduction rates were lower in mixed-species biofilms, but even then, better results were obtained in RNS-dominated conditions. According to the plasmabrush® PB3 manual, mainly nitrogen oxides are produced when operating with compressed air [70]. The selected system therefore appears to be suitable for the task and should deliver satisfactory results.

5.3. Limiting Factors of the Plasma Treatment

The influence of the buffering capacity of the sample on the effectiveness of the treatment was observed when tap water was exposed to plasma. As shown in Section 3.2.1, both the change in physical properties and the concentrations of NO_2^- and NO_3^- are influenced by water hardness. According to literature, the buffering effect is mainly caused by carbonic compounds, but can be also caused by ions and salts [43,64,71,72]. According to Simon et al., the antimicrobial effect of the plasma-treated water correlates with the formation of nitrous acid from the dissolved nitrogen oxides generated by the plasma. The authors show in [72] that, although the concentration of species measured after treatment can be relatively similar in water samples of different origin, the concentration of nitrous acid, and thus, the antimicrobial efficacy can vary. The slight differences in physicochemical properties of the tap water samples with different degrees of hardness, summarized in Table 3, did not lead to a significant reduction in the viability of *Pseudomonas*. Taking

into account the results from [72], this seems plausible as both water samples have the same origin—the sample with the higher degree of hardness was hardened as described in Section 2.3. The added substances (mainly CaCl_2 , MgCl_2 , and NaHCO_3), therefore, appear to have little influence on the antimicrobial effect of the plasma treatment in our case.

The antimicrobial effect was reduced even more when reused water was treated. The reused water sample contains, apart from suspended particles and sediments, an abundance of chemicals (alkalis, complexing agents, dispersants, surfactants, etc.) that can act as scavengers. Scavengers are substances which react with short-lived reactive species formed in the plasma, thereby reducing their concentration. Due to their high reaction rates, scavenging substances such as superoxide dismutase or catalase are often used in diagnostics to determine the working agent responsible for the antimicrobial effect [26,43]. However, the plasma–liquid interaction is often so complex that it is difficult to interpret the results of experiments performed in a controlled environment with deliberately added scavengers [26]. Then, the antimicrobial effect is also reported to depend on formation of peroxyxynitrate [40,73] or a mixture of nitrites, peroxides and acids [74]. It changes with gas composition [75], reactor design [40], and also with the surface-to-volume ratio according to [33,76]. According to the above, it appears that a large proportion of the reactive species produced during the treatment time studied (max. 30 min) is “consumed” by the chemicals in the sample without affecting the viability of the *Pseudomonas*. Also taking into account the possible biological contamination of reused water from a car wash (which was reduced here by autoclaving), it seems even more difficult to find the exact cause of the impaired efficacy of the treatment and is a topic for future studies.

5.4. Estimation of Additional Costs Due to the Use of Plasma

Another point that needs to be discussed in the context of an industrial application is energy efficiency and the associated costs. The following cost estimate only takes into account the additional costs incurred by a plasma system, whereby the car wash is assumed to be equipped with an air compressor. The values used are summarized in Table 4. The chosen plasma system consumes a power of about 1.2 kW [77]. Taking into account that a reduction of 1 log level can be achieved with clear tap water in about 10 min (compare Figure 4) and that such a reduction is sufficient to prolong the cleaning interval of a car wash, the treatment of a tank with a volume of 1000 L in a water circulation system of a car wash would take about 33 h, resulting in a electricity consumption of 40 kWh. The energy cost would amount to around 12 EUR, assuming an average price of electricity in the region of 0.30 EUR/kWh. If one then assumes a service life of 1000 h for consumables of the plasma system [78] and a price of 325 EUR per nozzle [79], and also takes into account the maintenance of an air compressor, around 13.5 EUR have to be added to the energy costs. A car wash consumes between 100 L and 200 L per car, so the plasma treatment with the chosen system would increase the total cost for a customer by around 3.80 EUR. This would correspond to an increase of approximately 25%, assuming an average price of 15 EUR per wash. Such a price increase may be acceptable for an environmentally conscious customer, but most likely not for the average customer. Given the results of the direct treatments and taking into account the results of the indirect treatment tests from Section 4, which showed that DI water can still act antimicrobially 3 h after plasma treatment, it appears that costs could be noticeably reduced if DI water were used instead of tap water. This is possible because deionizers are often installed in car washes (the chemicals work better with deionized water). If this is the case, the additional operating costs estimated above would not change, as the amount of DI water produced by the deionizer can remain unchanged. A different, more efficient reactor design or plasma system could also be used to reduce operating costs. According to Malik, who investigated the energy efficiency of various

plasma reactors for water purification, the efficiency of a pulsed streamer spark discharge is average [34]. The efficiency can be improved by maximizing the contact area of the plasma with the sample by bubbling air, oxygen, or the exhaust gases from the reactor through the treated sample. Apart from that, the author also mentions other types of discharges, such as pulsed corona or dielectric barrier discharges, which make water treatment more energy efficient. Here, too, efficiency can be increased even more by bubbling or spraying of the treated liquid, as reported by others [33,80]. Nonetheless, even if more efficient reactors using the above-mentioned discharge types emerge onto the market [81–83], they are usually not suitable for treatment of large water quantities.

Table 4. Overview of the assumed and calculated values of the cost estimate.

Reduction Level	Power Consum. [kW]	Water Volume [L]	Treatment Time [h]	Electricity Price [EUR/kWh]	Energy Cost [EUR]	Service Life [h]	Maintenance Cost [EUR]	Water Consum. [L/wash]	Total Cost Increase [EUR/wash]
1 log	1.2	1000	33	0.3	12.0	1000	13.5	150	3.8

5.5. Factors to Consider Before Industrial Application

Considering the costs, energy consumption, and results obtained, this means that the system used in this study is not suitable for the direct treatment of reused water. Based on the results obtained in this work, it appears that a blend of plasma-activated DI water and reused water would be the best solution for the example discussed and plasma system chosen. In addition, the bactericidal remanence effect of the plasma-treated deionized water shown in Figure 7 would contribute to a more energy, and thus, cost-efficient treatment of large quantities, as is common in industrial applications. Nevertheless, much more attention should be paid to the chemistry before a possible application. The remanence effect of plasma-treated DI water may be reduced if it is mixed with reused water containing oxidizable substances. Even though studies have shown that PAW is not toxic to mice [84], the formation of nitrates and nitrites in the water as a result of plasma treatment can pose a health risk in excessive quantities. For this reason, there are strict regulations regarding the permissible quantity, especially for drinking water [85]. Additionally, the reaction of the plasma with the chemicals in the reused water may produce other environmentally harmful substances. Excessive acidification of water is most likely harmful to the vehicle components. Then, the plasma not only generates reactive species, but also UV radiation. UV radiation mainly affects surfactants, but complexing agents can also release substances under the influence of UV. In the case of dispersants, at least an influence on the speed of the partner exchange is to be expected, whereby the reactions are often difficult to reverse [86,87]. These points have to be clarified before an actual industrial application.

6. Conclusions

With a view to industrial feasibility, the suitability of an commercial off-the-shelf plasma system for antibacterial water treatment was tested in this work. A car wash was chosen as an application example and *Pseudomonas aeruginosa* was used as a test germ. The results obtained with DI water in terms of physicochemical properties were in good accordance with previous work. The best inactivation results of at least 6 log levels after 20 min of direct plasma treatment were also achieved with this water type. As expected, significantly higher cell viability was observed in clear tap water samples with different degrees of hardness. The lower antimicrobial effect of the plasma treatment was attributed to the buffering capacity of the tap water. In contrast, the change in the degree of hardness led to an insignificant change in cell viability. This seems plausible as the samples have the same origin. It also suggests that the substances used to harden the water had little

influence on the chemistry occurring at the plasma–liquid interface. The bacterial load remained almost unchanged when the reused water samples were treated. This can be attributed to the presence of chemicals that most likely act as scavengers, and thus, they significantly reduce the concentration of reactive species generated by the plasma. In a series of indirect treatment tests, a strong and long-lasting bactericidal remanence effect of plasma-activated DI water was found, which can shorten the required treatment time in a potential application, and thus, increase the energy and cost efficiency of the process. Taking into account the operating costs, the results achieved and the conditions prevailing in a car wash, a mixture of plasma-activated DI water and reused water appears to be the best solution for a possible application of the selected plasma system in the example discussed. Nevertheless, due to the complex composition of the reused water in a car wash, several open technical and legal questions still need to be clarified before an actual application. As a first step, future work should concentrate on analyzing the chemical composition of the reused water and the interaction of the substances it contains with the plasma. At the same time, the possibilities for increasing the efficiency of treatment, which currently appears to be the main limiting factor, should also be researched.

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Abbreviations

The following abbreviations are used in this manuscript:

WHO	World Health Organization
DBP	Disinfection by-products
EC	Electrical conductivity
ORP	Oxidation reduction potential
CRB	Chlorine-resistant bacteria
AOP	Advanced oxidation process
RCS	Reactive chlorine species
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RONs	Reactive oxygen-nitrogen species
PAW	Plasma-activated water
DI	Deionized
°dH	degrees of German hardness
MGDA	Methylglycinediacetic acid trisodium salt
TSA	Tryptic soy agar
TSB	Tryptic soy broth
CASO	Casein-soy flour-peptone agar
CFU	Colony forming unit

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