Antimicrobial Effect of Plasma-Activated Tap Water on *Staphylococcus aureus, Escherichia coli,* and *Candida albicans*

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Abstract: In this study, the potential antimicrobial activity of plasma-activated tap water (PAW) was evaluated against *Staphylococcus aureus, Escherichia coli,* and *Candida albicans*. For this, PAW was prepared in a gliding arc plasma system using two treatment conditions: stagnant water and water stirring by a magnetic stirrer, called moving water. Subsequently, their oxidation-reduction potential (ORP), pH, electrical conductivity (σ), and total dissolved solids (TDS) were monitored in different areas of the sample divided according to the depth of the beaker. It was observed that PAW obtained in dynamic conditions showed a more uniform acidity among the evaluated areas with pH 3.53 and ORP of 215 mV. Finally, standardized suspensions of *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 10799), and *Candida albicans* (SC 5314) were treated with PAW, and the reduction of viable cells determined the antimicrobial effect. Our results indicate that the tap water, activated by plasma treatment using gliding arc, is an excellent inactivation agent in the case of *Staphylococcus aureus* and *Escherichia coli*. On the other hand, no significant antimicrobial activity was achieved for *Candida albicans*.

Keywords: plasma activated water; tap water; atmospheric plasma; gliding arc discharge; *Staphylococcus aureus*; *Escherichia coli*; *Candida albicans*

1. Introduction

In recent years, plasma-activated water (PAW) has gained prominence due to its application in medicine [1–3], mainly due to the effects of inactivation in microbial species caused basically by the stimulation of high biochemical and biological activities that alter the properties of water due to its exposure to plasma [4–8]. This plasma water activation technique is inexpensive and environmentally friendly since it is free of chemicals [9]. For this type of application, atmospheric non-thermal plasmas (ANTP) are used, namely, the dielectric barrier discharge (DBD) or the gliding arc plasma jet (GAPJ) [10–17]. The plasma used as a water activating agent can be applied directly submerged in the liquid or applied to millimeters of the liquid's surface [18–24].

According to Zhou et al. [8], the plasma activates a mixture of reactive oxygen and nitrogen species (RONS) in water that is highly effective in the long term against resistant fungi, bacteria, and viruses. Therefore, RONS are primarily responsible for microbial inactivation, making PAW a potential candidate for application in clinical practice. The RONS generated in the exposure of water to plasma can include types of species with long life (between years and tens of minutes); namely, ozone (O_3), hydrogen peroxide (H_2O_2), nitrates (NO_3^-), and nitrites (NO_2^-). Also, short-lived species (between seconds and nanoseconds) are generated, namely, peroxynitrite ($ONOO^-$), peroxynitrite ($OONO_2^-$), superoxide (O_2^-), nitric oxide ($NO\bullet$) and hydroxyl radicals ($\bullet OH$) [8,13]. Despite the good antimicrobial effect of PAW, it is essential to note that these reactive species can be dangerous to human health, and their activity in eukaryotic cells must be considered before any application. Recently, Han et al. [25] applied cold plasma directly to edible films to investigate subacute oral toxicity and demonstrated low toxicity without generating harmful by-products to human health. Borges et al. [26] showed that the direct use of a cold plasma jet on epithelial cells has low toxicity. Finally, Ibis and Ercan [27] evaluated the in-vitro toxicity of distilled water activated by plasma and, later, nebulized on healthy eukaryotic cells of the human tracheal epithelium where they demonstrated that there was no damage significant to eukaryotic cells. Therefore, it is evident that the application of plasma directly to the human cell, the most aggressive treatment method, causes low toxicity. On the other hand, recent studies have shown that there is no toxicity of PAW on healthy human eukaryotic cells.

Thinking about the construction of a bench device for water activation/treatment, which can be used in gynecological, dental, and medical clinics, it is more advantageous to use plasma applied to millimeters from the surface, which facilitates the architecture of building a device, without lose the effectiveness of inactivation of microbial species [8–17]. However, despite PAW's tremendous technological appeal, mainly due to its potent antimicrobial effects and wide application in clinical practice, some technical challenges need to be overcome. As an example, increasing the concentration of RONS produced.

As reported in the literature, the amount of RONS generated per unit volume of activated water is generally low, requiring a long time of exposure to plasma (several minutes or hours) to reach a sufficient amount of activated water [28–32]. Besides the limited volume of activated water, other factors inhibit the fabrication of portable equipment for clinical use. For example, the use of pure gases, such as oxygen, argon, and/or helium, in ANTP reactors [2,30–34] can be expensive, demanding high voltages to maintain the electrical discharge, in addition to having low efficiency in the production of RONS in high volumes. Another point that contributes to the increase in the costs of the process is the need for deionized or distilled water to control and standardize the water. To overcome these challenges, a versatile and low-cost PAW process is required. In this sense, air plasma, i.e., an electrical discharge that generates high-density plasma in the air [7,29,35–37], is an attractive source of low-cost and high-efficiency RONS. In this context, the forward vortex flow reactor (FVFR) emerges as a gliding arc (G arc) source that potentially has all requirements mentioned above [38,39]. Another fundamental point is the exchange of deionized or distilled water for tap water. This exchange is essential to reduce PAW's manufacturing costs, as these water purification processes require expensive equipment. Although tap water has different properties in each country, state, city, and even between neighborhoods, it is still a cheap and easily accessible alternative around the world. Even without a universal standardization of its properties and characteristics, tap water can be activated by plasma and used in medicine, agriculture, etc. In fact, this is done after a study of toxicity in living beings. Recently, PAW has become a frequently employed antimicrobial agent [24]. The processes that involve it can be divided into two types: the direct-PAW mode and the indirect-PAW mode. In the first, microorganisms present in the water are inactivated during the plasma treatment of the liquid. In the second case, the water previously treated by plasma is used to induce antibacterial/antifungal activity immediately after its generation or after some time. An exciting feature is that the

PAW when adequately stored, can retain its antibacterial/antifungal properties for several days [40–42]. This long-term antimicrobial efficacy [42,43] can help to inactivate various microbiological species in regions far from the source of water activation, thus expanding PAW applications in areas far from large economic centers.

This experimental study is outlined as follows: first, the experimental setup is introduced based on the plasma jet generated in an FVFR-G arc reactor using compressed air placed a few millimeters from the surface of the tap water. The PAW produced by this device has been stored for later use. Two different modes of water treatment were tested: in the first case with stagnant water and in the second case with moving water with the aid of a magnetic stirrer. After plasma processing, the antimicrobial properties of the treated water were evaluated against *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 10799), and *Candida albicans* (SC 5314). Finally, the impact of individual plasma parameters on tap water properties were discussed and their influence on PAW antimicrobial properties were assessed.

2. Materials and Methods

2.1. Experimental Configuration for Obtaining PAW

PAW was prepared by a gliding arc plasma system schematically shown in Figure 1. The experimental setup comprises a plasma reactor, a high-voltage power supply, an oscilloscope, an optical emission spectrometer and, an infrared camera. The gliding arc plasma was generated in a forward vortex flow reactor (FVFR) type [39], and the outer region is composed of plasma plume and post-discharge regions [39,40]. The gas used in this study was air generated by an air compressor (Schulz CSD 9/50, Joinville, SC, Brazil) with a flow of 5 L min^{-1}. This air flow was chosen due to the formation of a continuous gliding arc discharge with the lowest possible flow, as demonstrated by Doria et al. [39]. The system was powered by a high-voltage transformer (Linsa, Indústria Eletro Mecânica Linsa LTDA, São Paulo, SP, Brazil) operating at 7.5 kV and 60 Hz. A high-voltage resistance (approx. 1 kΩ) was used to protect the transformer in case of an electric arc. A Variac transformer (VARIAC, Cleveland, OH, USA) was used to adjust the high voltage of the plasma reactor.
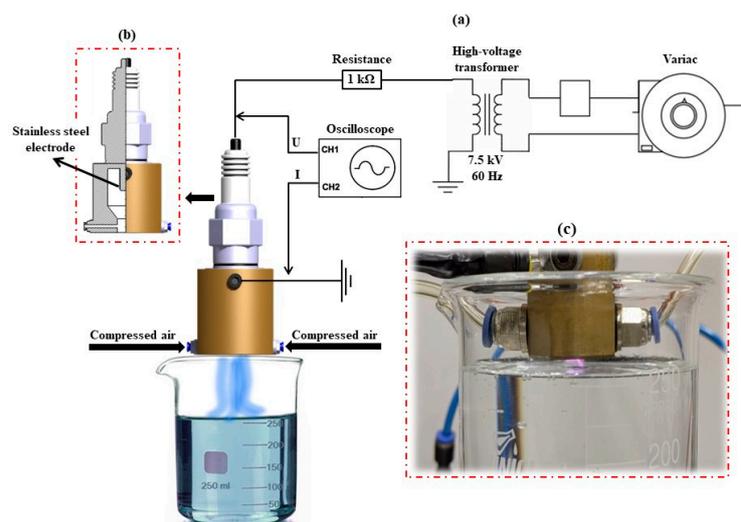
Figure 1. (**a**) Experimental setup, (**b**) reactor, and (**c**) photograph of the FVFR G-arc reactor for treatment of tap water.

A 250 mL beaker containing tap water collected at the Laboratório de Plasmas e Processos (LPP—23°12′31.2″ S 45°52′40.9″ W) (pH of 6.53 and with a soft hardness of 2.0 mg/L CaCO$_3$) (dataset obtained in São José dos Campos—São Paulo—Brazil according to the National Institute of Metrology, Standardization and Industrial Quality, Inmetro [44]) was placed on a magnetic stirrer (Kasvi CK FG, Kasvi, São José dos Pinhais, PR, Brazil)

and the nozzle of the gliding arc reactor was positioned 5 mm from the water surface. The water treatment time was 30 min.

To obtain the electrical signals from the gliding arc discharge, a high voltage probe (Tektronix P6015A, Tektronix, Beaverton, OR, USA) and a self-adjusting current probe (Agilent N2869B, Agilent, Santa Clara, CA, USA) were used. All electrical signals were recorded on a digital oscilloscope (Keysight DSOX1202A, Keysight, Santa Rosa, CA, USA), and the current signal was inferred directly from the grounded electrode. A photo of the discharge configuration (Figure 1c) was taken with a smartphone (Google Pixel 3XL, Google, Mountain View, CA, USA).

Doria et al. [39] reported an increase in the error measured for the discharge power due to the fact that some charged particles escape from the plasma plume. Schmidt et al. [24], related the same problem using distinct plasma reactors. Based on this problem, to calculated the electrical power dissipated into the plasma (P_{dissip}), Doria et al. [39] suggested using the following equation:

$$P_{dissip}(W) = \frac{1}{T_2 - T_1} \int_{T_1}^{T_2} V(t)I(t)dt \tag{1}$$

where $V(t)$ is voltage, $I(t)$ is the electric current and $T_2 - T_1$ is the time interval.

Figure 2a shows the typical waveforms for discharge voltage and current recorded by oscilloscope using a fixed Variac control position. As can be seen, the peak-to-peak voltage is 2694 V for air flow of 5.0 L min^{-1}.

(a)

(b)
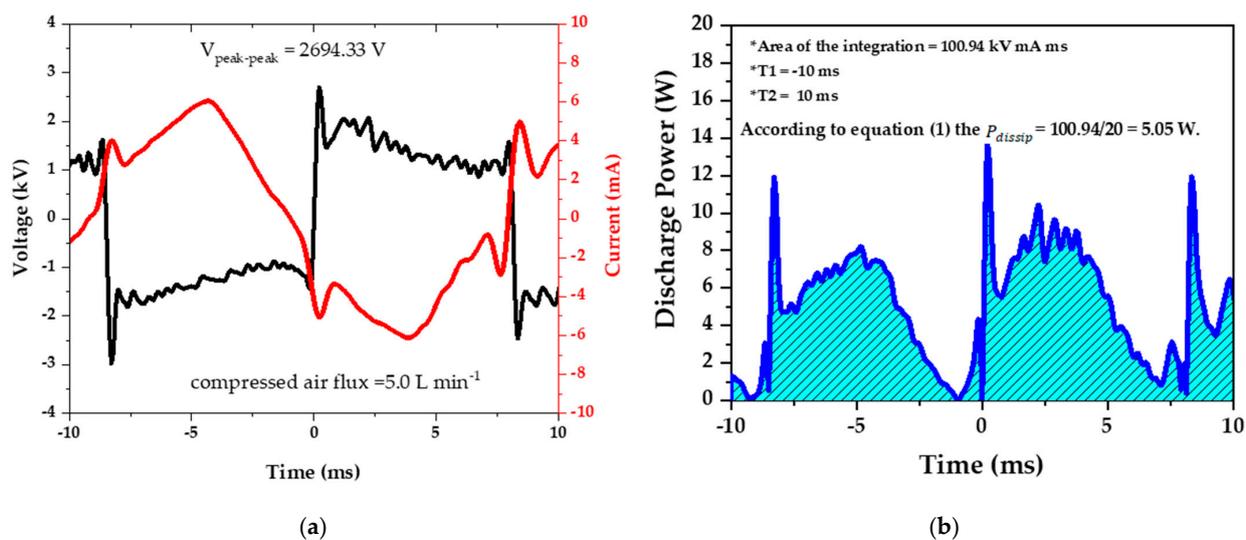
Figure 2. (**a**) Discharge voltage and current waveforms of G-arc operating with air flow of 5 L min^{-1} and (**b**) waveform of G-arc discharge power used to calculate the mean power through Equation (1).

Figure 2b illustrates the instantaneous discharge power waveform as a function of time. It is worth highlighting that the integral of the curve over a time interval ($T_2 - T_1$) of 20 ms allows to obtain a mean power in the discharge of 5.1 W. As a consequence, the consumption of 10 Wh for the production of 1 L PAW is equivalents to a nowadays expense of below 0.01\$ or 0.01€ per liter for electricity and water. Therefore, the choice of compressed air gas is justified.

Optical emission spectroscopy (OES) was used to characterize the main plasma species in UV-visible range of 200–500 nm. For this, an optical emission spectrometer (Ocean Optics USB4000, Ocean Insights, Rockster, NY, USA) with a resolution of 1.5 nm was used. The OceanView software (OceanView Software, Ocean Insights, Rockster, NY, USA) was used to acquire the optical spectrum. As shown in Figure 3, the optical emission spectrum of the air plasma contains NH, N_2, and OH species, which come from atmospheric air [39].
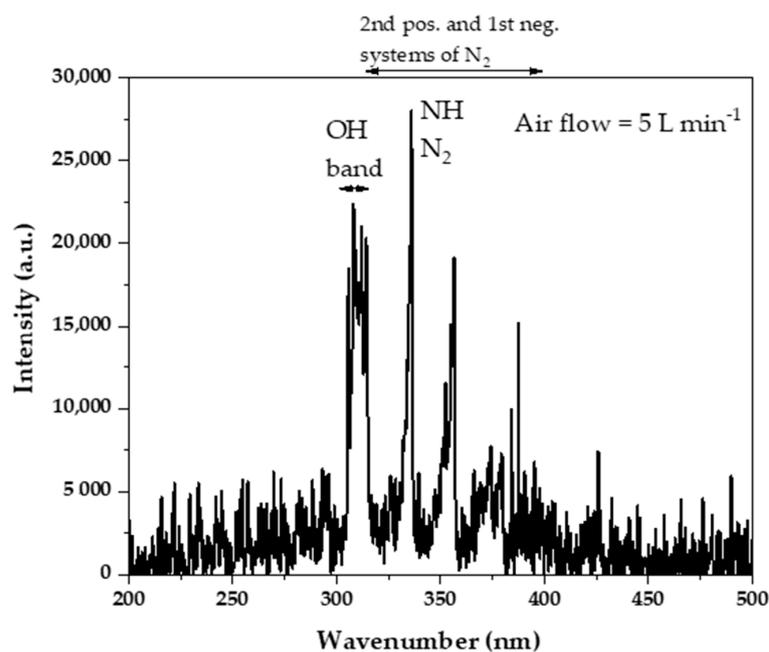
Figure 3. OES spectrum of gliding arc plasma jet operating at air flow of 5 L min^{-1} and discharge power of 5.1 W.

It is worth mentioning that both the electrical and the optical emission parameters presented aimed at detail the plasma used to treat the tap water. As the work is focused on the changes generated in tap water and its antimicrobial effect, we find it more convenient to present these data in this section instead of showing them in results and discussions.

Finally, thermal images of the water during plasma treatment were taken using an IR camera (model TiS 10, Fluke, Everett, WA, USA).

2.2. Water Physicochemical Properties Measurements

The pH value, the oxidation-reduction potential (ORP), the electrical conductivity (σ), the total dissolved solids (TDS), as well as the water temperature were measured with a multiparameter (Mult-007, IonLab, Araucária, PR, Brazil), right after the plasma activation process. For the evaluation of the physical-chemical parameters, the experiment was designed as follows. Tap water was placed in a 250 mL beaker on a magnetic stirrer, and two forms of treatment were performed. In the first case, the tap water was exposed to the plasma with the agitator turned off, and in the second case, the agitator was kept on at 200 rpm. Tap water before plasma activation was used as a control sample. Firstly, the effect of treatment time in stagnant and stirring water modes was studied with the multiparameter water sensors (Multisensor, IonLab, Araucária, PR, Brazil) positioned at the bottom of the beaker. For this step, tap water samples (250 mL) were treated between 5 to 30 min with water samples divided into six sets corresponding to different plasma exposure times (5, 10, 15, 20, 25 and 30 min) for each mode. It is essential to mention that to maintain the same activation kinetics of PAW, tap water (250 mL) was changed for each time of exposure to plasma (5, 10, 15, 20, 25 and 30 min). This methodology was repeated for sets of samples every 5 min for both forms of water treatment. It is worth mentioning that, for each sample, the measurements were repeated five times.

The second part of the study aimed to evaluate the variation of the PAW physicochemical parameters along with the depth of the beaker right after the plasma treatment for the stagnant and agitated water modes. Figure 4 illustrates the sensors' positions at different points in the beaker used to measure the physicochemical parameters of the PAW.
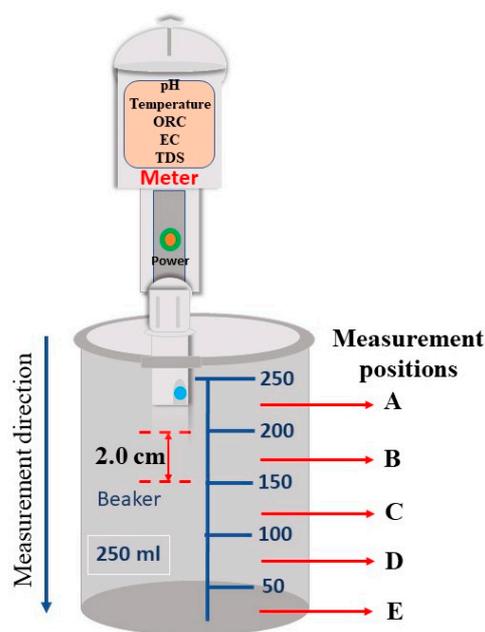
Figure 4. Water characterization points before and after plasma treatment using the multi-parameter meter as a function of depth.

A gradient of the RONS content along with the depth of the beaker can be expected with a higher concentration near the arc nozzle G and a lower concentration at the bottom of the beaker. Measurements were performed in five vertically aligned positions defined below: position A (between 250 and 200 mL), position B (between 200 and 150 mL), position C (between 150 and 100 mL), position D (between 100 and 50 mL) and position E (between 50 and 0 mL). Also, an investigation of the evolution of the PAW physicochemical parameters was carried out. The methodology used in both water treatment modes consisted of measuring water characteristics at three different times after treatment: the first data acquisition was 5 min after treatment, the second measurement 2 h after treatment, and the third measurement, which occurred 24 h after treatment. It is worth mentioning that the PAW was kept at room temperature (25 °C) in the beaker, and the measurements were repeated five times.

2.3. Microbiological Assays

2.3.1. Strains and Inocula Preparation

Reference strains of a Gram-positive bacterium *Staphylococcus aureus* (ATCC 6538), Gram-negative bacterium *Escherichia coli* (ATCC 10799), and the fungus *Candida albicans* (SC 5314) were included in this study. The strains were plated on tryptic soy agar (TSA), Broth Heart Infusion (BHI), and Sabouraud dextrose, respectively. Plates were incubated at 37 °C for 24 h, under aerobiosis. Then, standardized suspensions containing 10^6 cells/mL of each microbial species were prepared in sterile saline solution (NaCl 0.9%) with the aid of a spectrophotometer (AJX-1600, Micronal, São Paulo, SP, Brazil), according to the following parameters: wavelength (λ) of 490 nm and optical density (O.D.) of 0.374 for *S. aureus*, $\lambda = 600$ nm and O.D. = 0.050 for *E. coli*, and $\lambda = 530$ nm and O.D. = 0.138 for *C. albicans*.

2.3.2. Antimicrobial Activity

For the evaluation of antimicrobial activity, the groups tested were: (i) PAW (pH 3.5); (ii) Non-activated tap water (TW) (negative control, pH 6.5); and (iii) Sterile distilled water adjusted to pH 3.5 (low pH control). The low pH control was included as a control to evaluate the possible effect of the low pH on the microorganisms. PAW was sterilized by filtration using a 0.22 μm membrane (Biofil Syringe filter, Microlab Scientific Co., Mongkok, Kowloon, Hongkong), with the aid of a sterile syringe. An aliquot of 125 μL of the

microbial suspension was added to 875 μL of PAW in microtubes, homogenized, and maintained for 10 and 30 min. Afterward, serial dilutions of the suspension were obtained in sterile saline solution (NaCl 0.9%). An aliquot of 100 μL were plated on tryptic soy agar (TSA) for *S. aureus*, Broth Heart Infusion (BHI) for *E. coli*, and Sabouraud dextrose for *C. albicans*, according to the method described by Miles et al. [45]. Plates were incubated at 37 °C for 24 h, under aerobiosis. After the incubation period, the number of colonies was counted, and the value of colony-forming units per milliliter was calculated (CFU/mL). The experiments were carried out in triplicate.

2.3.3. Statistical Analysis

Graphpad Prism v7.0 software (Graphpad Company, San Diego, CA, USA) was used to perform the statistical analysis and plot the graphs. Data was previously analyzed by the normality test. Results on antimicrobial effect were compared by One-way Analysis of variance (ANOVA) and Tukey's post hoc test for the *S. aureus* and *C. albicans* groups. As data obtained for *E. coli* was not normally distributed, they were compared by Kruskal-Wallis and Dunn's post hoc tests. The level of significance was set at 5%.

3. Results and Discussion

3.1. Physicochemical Measurements and Thermal Analysis of Water

It is worth mentioning that the water collected from different taps in the same building did not present other properties or parameters. In this case, it was not necessary to use an error bar in physicochemical parameters. Even so, in buildings with older pipes or with the possibility of taps with little use, there is probably some variation in the water parameters, basically due to the fact that the water line is slightly older. In such cases, better attention will be required to the collected parameters.

3.1.1. Effect of Treatment Time

Figure 5a show the pH and ORP measurements as a function of the treatment time for the conditions of stirrer turned off and stirrer turned on during the plasma-activated water. It is important to note that the measurements were performed at the bottom of the beaker.

(a)   (b)
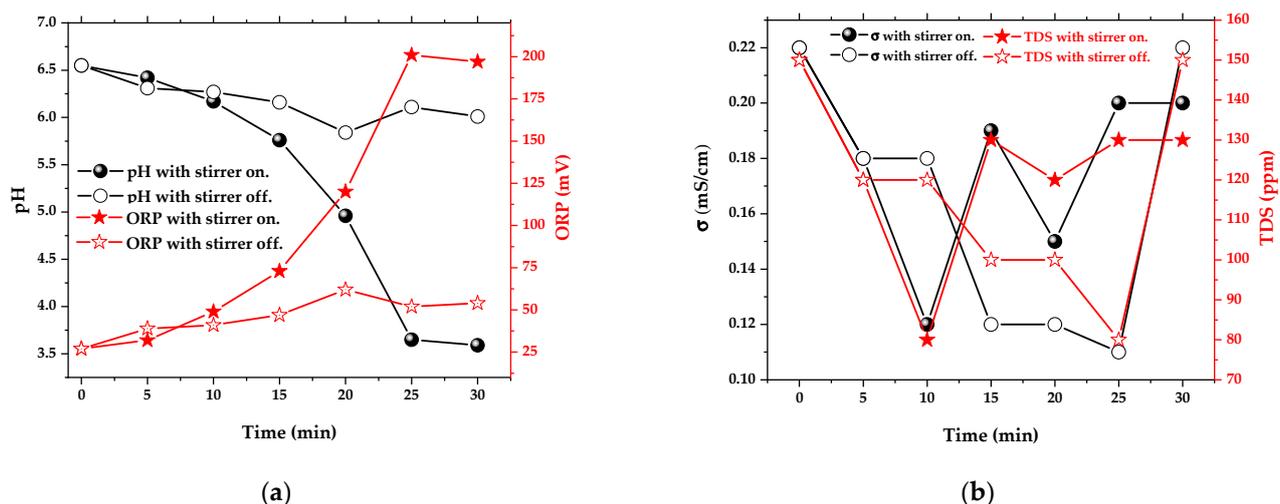
Figure 5. (**a**) pH and oxidation-reduction potential (ORP) of water as a function of plasma treatment time for conditions of stirrer on and off during the plasma activation. (**b**) electrical conductivity and total dissolved solids of water as a function of plasma treatment time for conditions of stirrer on and off during the plasma activation (error bar is not shown in the graphs (pH error (±0.05); ORP error (±5 mV); σ error (±5 μS/cm); TDS error (±5)).

It can be noted here a different behavior between the stagnant water and water stirring by a magnetic stirrer, both for pH and for ORP. When the plasma jet is placed a few millimeters from the water surface and treatment is started on 250 mL of stagnant

water, there is a slow diffusion of RONS species through the water volume, making it difficult for these species to migrate to the bottom of the beaker, this translates into a slow reduction in pH (6.53 ± 0.05 to 6.00 ± 0.05) and an increase in ORP (26 ± 5 to 52 ± 5 mV) at the beaker's bottom. This fact is not observed, however in the case where the water remained agitated throughout the treatment. In this case, there was a drastic decrease with subsequent saturation of the pH value after 25 min of treatment. For the ORP, there was an exponential increase in its value during the 25 min of treatment and subsequent saturation at 200 ± 0.05 mV. These results demonstrate the importance of stirring large amounts of water during water treatment with a gliding arc plasma jet in non-contact mode. In a recent study, M. Schmidt et al. [24] used an Inductively-Limited Discharge to activate 500 mL of tap water and, after 30 min, reached a pH = 6. F. Judeé et al. [12] used a DBD system for tap water treatment and a pH reduction from 7.5 to 6.5 was observed. Thus, the use of the gliding arc system with moving water proved to be a very efficient activation treatment for tap water.

Due to the lack of studies with tap water using air as a gas plasma source, as a comparison, studies that investigated the ORP of sterile distilled water (SDW) were used. According to Q. Xiang et al. [46,47], the ORP increased from 546 to 552 mV after the G Arc treatment of 200 mL of SWD at discharge power of 750 W and a compressed air flux of 30 L min^{-1} for 30 s without stirring. Zhao et al. [48], using an atmospheric cold plasma jet (ACPJ), treated 30 mL of SWD for 5 min in static mode. They observed an increase in PAW's ORP value to 546.77, 558.57, and 565.40 mV at 15, 22, and 30 kV, respectively, showing that the voltage had a significant impact on the oxidation-reduction potential. On the other hand, D. Cheng et al. [49], using an atmospheric-pressure plasma jet (APPJ), observed an ORP increase from 224.7 to 463.8 mV in SWD after 20 min of plasma treatment and with a magnetic stirrer. These results corroborate our data (Figure 5a) and show the importance of stirring in the PAW process.

Figure 5b show the σ and TDS measurements as a function of the treatment time for the stirrer's conditions turned off, and stirrer turned on during the plasma-activated water. As demonstrated for pH and ORP measurements, it can be stated that a different behavior as a function of plasma treatment time occurs between the stagnant water and water stirring by a magnetic stirrer, both for σ and TDS. Figure 5b shows that during the first 25 min of activation, σ decreases. For plasma activation time greater than 25 min, the electrical conductivity increases and abruptly returns to the tap water value before the activation, i.e., 220 ± 5 μS/cm. In the case of stirrer on, during the first 10 min of activation, σ decreases from 220 ± 5 to 115 ± 5 μS/cm. For plasma activation time higher than 10 min, σ increases more slowly to the value of 220 ± 5 μS/cm in comparison with stagnant water experiment. F. Judeé et al. [12] used a DBD system for tap water treatment. They observed similar behavior with a decrease from 647.33 to 614 μS/cm in the first 5 min. For 5 and 30 min of plasma exposure, they found that σ increased linearly with activation time, in a range covering values from 614 to 731.33 μS/cm. M. Schmidt et al. [24] found that after 30 min of tap water plasma treatment, they achieved an increase in σ of less than 50 μS/cm (from ~600 to 650 μS/cm). In the case of TDS measurements, the behavior was the same of σ (as can be seen in Figure 5b) due to the direct relationship between dissolved solids in the liquid and conductivity.

3.1.2. Effect of Water Dynamic Plasma Activation

Table 1 shows the physicochemical parameters of the PAW along the depth of the beaker after plasma treatment for both modes. The pH, ORP, σ, TDS and temperature in both modes were measured after 5 min, 2 h and 24 h of plasma treatment. During all characterization, the PAW was kept at room temperature (25 °C) in the beaker. As shown in Table 1, the stagnant tap water activated by plasma has a reduced diffusion across the volume of water, making it difficult for these species to migrate to the bottom of the beaker. At 5 min, a gradient of temperature of 43 ± 1 in position A to 37 ± 1 °C in position E was observed. This heating effect was expected due to the proximity of the plasma jet

to the water surface. For this same analysis time, it was observed that in position A the pH = 2.77 ± 0.05, but at the bottom of the beaker (position E) the pH = 6.05 ± 0.05, showing the difficulty of activation of the entire liquid volume when the water is kept static. In turn, the ORP at the top of the PAW (position A) has approximately 6 times higher value when compared to the ORP at the lowest position (position E). Both the σ and TDS exhibit the same behavior as ORP.

Table 1. Measurements of the physicochemical parameters of the non-treated tap water and PAW in both modes realized at 5 min, 2 h and 24 h after the activation by plasma (according to the equipment manual, all measured parameters have an error of ± 2%).

| Time after PAW	Sensor Position	pH (±0.05)	Temperature (±1 °C)	ORP (±7 mV)	σ (±5 μS/cm)	TDS (±5 ppm)
			Control sample			
-	A to E	6.55	25	27	220	150
			PAW sample—Stagnant water			
	A	2.77	43	239	540	370
	B	2.80	43	237	480	330
5 min	C	3.40	43	217	400	280
	D	5.55	39	75	280	190
	E	6.05	37	51	220	150
	A	3.33	25	212	240	160
	B	3.98	25	167	230	152
2 h	C	3.96	25	165	220	150
	D	3.95	25	165	220	150
	E	3.96	25	165	220	150
	A	3.85	25	183	220	150
	B	3.85	25	183	220	150
24 h	C	3.85	25	183	220	150
	D	3.85	25	183	220	150
	E	3.85	25	183	220	150
			PAW sample—Stirred water			
	A	3.21	34	221	210	80
	B	3.33	33	212	210	90
5 min	C	3.32	33	211	220	150
	D	3.31	32	211	220	150
	E	3.48	32	199	220	150
	A	3.53	25	215	220	150
	B	3.53	25	215	220	150
2 h	C	3.53	25	215	220	150
	D	3.53	25	215	220	150
	E	3.53	25	215	220	150
	A	3.53	25	215	220	150
	B	3.53	25	215	220	150
24 h	C	3.53	25	215	220	150
	D	3.53	25	215	220	150
	E	3.53	25	215	220	150

Two hours after the plasma treatment, all measured parameters exhibit much better homogeneity. However, only 24 h after the treatment in static mode, the PAW parameters reached stability with the same values for all measured positions in the beaker. While the water temperature, conductivity, and TDS returned to their initial values, the pH factor and ORP still maintain values that are different from those of untreated water.

On the other hand, only 5 min after plasma treatment, the water stirred presents a quasi-stability of the physicochemical parameters of the PAW. After 2 h, the total homogeneity of all parameters of the PAW is achieved. Therefore, based on pH and ORP results, respectively, 3.53 ± 5 and 215 ± 7 mV, the PAW treated for 30 min in the dynamic mode was chosen as the best option for the inactivation of microbiological species investigated in this work.

According to the literature [24,50,51], the continuous decrease in pH of the plasma-activated nonbuffered solution (soft hardness of 2.0 mg/L $CaCO_3$ of our tap water) with increasing treatment time form new chemical species that be responsible for the decrease in the pH of water. However, as shown in Figure 5a the pH reaches a steady state after a certain activation period and remains constant. Another parameter that increased 10 times after the treatment was the ORP, which determines the ability of solutions to oxidize or reduce a substance. The ORP concerns the concentration of oxidizers and their strength or activity [50]. ORP is reported to be the principal responsible for destructing microbiological systems' membrane integrity with the fundamental function of affecting the cells' inner and outer membranes [52]. Among the RONS generated in PAW, hydrogen peroxide (H_2O_2) is mainly responsible for the formation of ORP [53] and has a rapid potential for disinfection of liquids [17,54,55].

3.1.3. Thermal Analysis of tap Water during Plasma Treatment

Figure 6 show the infrared thermal images of the beaker with water during the plasma treatment in stagnant and stirred modes, respectively. The thermal images of the beaker during the activation of the water by plasma were recorded in 0 min; 1 min; 5 min; 10 min; 15 min; 20 min; and 30 min, respectively. For stagnant water, a slow temperature gradient can be seen through the color gradient. This behavior can be compared to the difficulty of reactive species from plasma, such as RONS, in migrating to the bottom of the beaker, a fact that is correlated to the slow reduction in pH over the volume of water (as shown in Figure 5a). However, this fact is not observed in the case where the water remained agitated throughout the treatment. In this case, a continuous temperature gradient occurs, where, in 20 min of treatment, a continuum of the color gradient was observed which shows a thermal balance of the PAW contained in the beaker. This result resonates with the saturation of pH and ORP presented in Figure 5a (stirred on). Therefore, the thermal analysis of water during the plasma activation process demonstrates the importance of the effect of agitating large volumes of water during its treatment, especially for the case of non-contact plasma jet treatment.
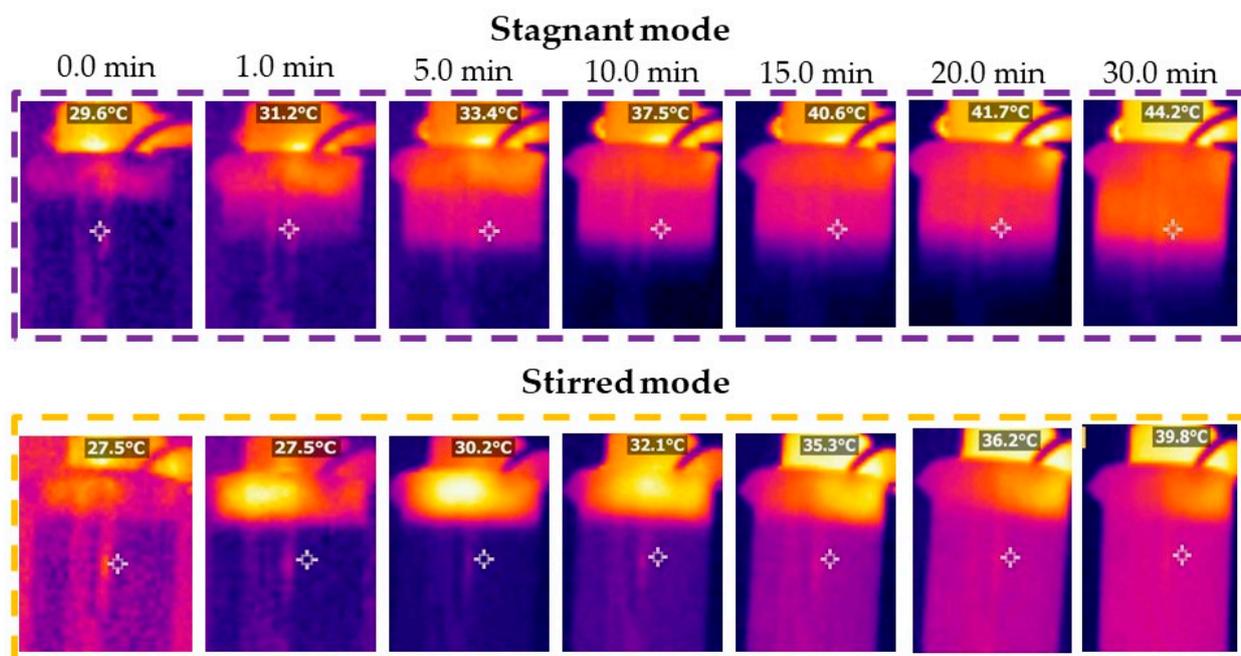

Figure 6. Thermal images of tap water during plasma treatment in stagnant and stirred mode: thermal image of the beaker during: 0 min; 1 min; 5 min; 10 min; 15 min; 20 min; and 30 min of plasma treatment.

3.2. Microbiological Analyses

To screen PAW antimicrobial activity, three clinically relevant microbial species, were included in the study. *Staphylococcus aureus* and *Escherichia coli* were included, as they are related to infections acquired in hospital environments [55] and represent the main groups of bacteria (Gram-positive and Gram-negative, respectively). *Candida albicans* are a species of opportunistic fungus that can cause various human diseases, from superficial to widespread infections [56]. Therefore, the inclusion of several microbial species in antimicrobial screening studies is vital due to the different cellular structures and metabolism that considerably affect susceptibility.

Figure 7 shows the results obtained for the effect of PAW in *S. aureus*. As can be seen, a significant reduction in viable cells was detected after contact with PAW for 10 min and distilled water pH 3.5 ($p = 0.0001$) when compared to non-activated tap water ($p = 0.0035$).

(**a**)

(**b**)
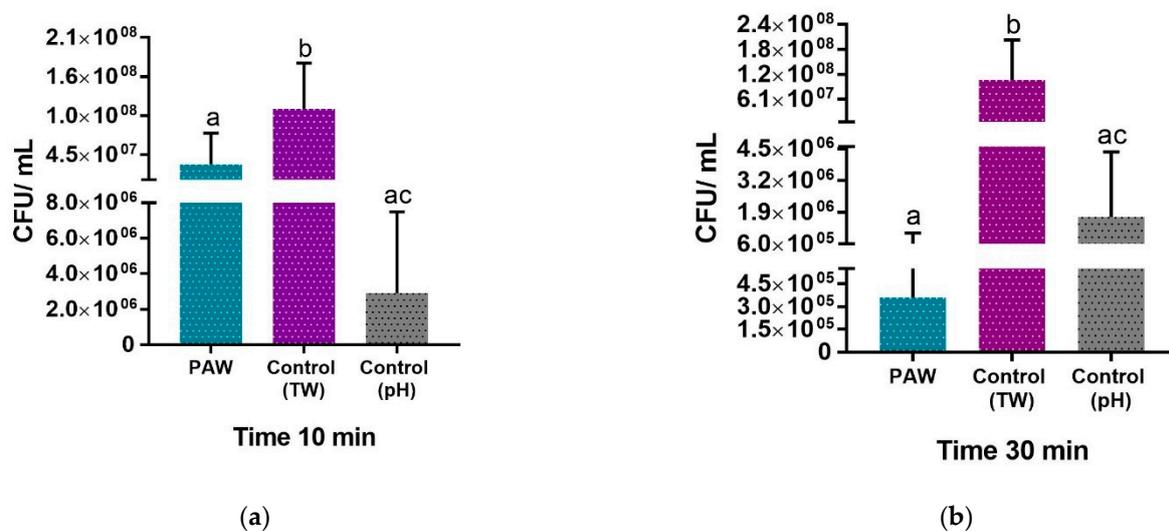
Figure 7. Mean and standard deviation of the number of *Staphylococcus aureus* viable cells (CFU/mL) after exposure to plasma-activated water (PAW) for 10 and 30 min. TW: non-activated tap water (negative control); Control (pH): distilled water pH 3.5. Different superscript letters indicate significant differences among the groups.($p < 0.05$) based on ANOVA statistical test followed by Tukey's post hoc test.

Exposure to PAW for 30 min showed the same results presented for 10 min, i.e., PAW and distilled water with pH 3.5 significantly reduced the viability of *S. aureus* when compared to the control ($p = 0.0014$ and 0.0016, respectively). Therefore, our results showed a reduction of 3-log UFC-mL^{-1}, which is similar to the result obtained previously by Zhang et al. [57]. However, these authors used deionized water exposed to argon and oxygen (2% Ar/O$_2$) and a dielectric barrier discharge plasma reactor (DBD) for 5 min. Also, the time of water exposure to plasma was longer than that used in our study (40 min). Pemen et al. [58] reported that PAW produced from tap water using a transient arc plasma reactor and a loop system could inhibit *Staphylococcus epidermidis* after 20 min of exposure. In the present work, PAW and distilled water with pH 3.5 showed a similar inhibition effect ($p > 0.05$) in *S. aureus*.

On the other hand, the PAW significantly reduced the number of *E. coli* after 10 min of exposure, both to non-activated tap water ($p = 0.0002$) and distilled water pH 3.5 ($p = 0.0498$) (Figure 8). Interestingly, after 30 min of exposure, no differences were detected between the groups to the control ($p > 0.05$). A previous study reported the effect of PAW on *E. coli* contained in a mixed suspension with *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*, where PAW was obtained after exposure of distilled water to the gliding arc discharge for 10 min, and *E. coli* was inhibited after 20 min of contact [59]. In addition, Traylor et al. [42] observed a significant reduction in *E. coli* after 15 min of exposure to PAW produced by the air dielectric barrier discharge.
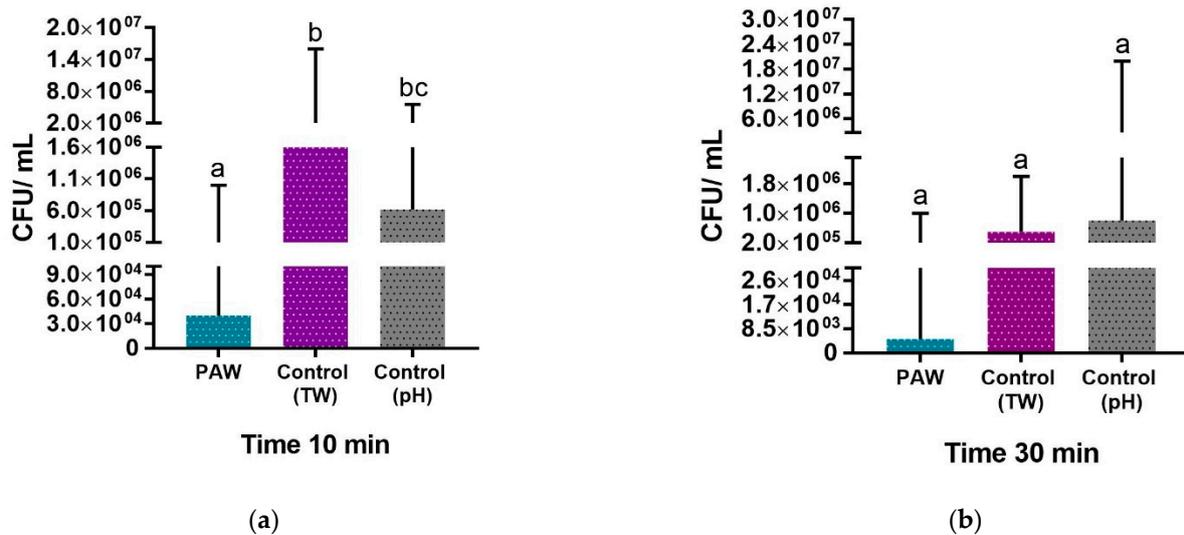
Figure 8. Median and range values of the number of *Escherichia coli* viable cells (CFU/mL) after exposure to plasma activated water (PAW) for 10 and 30 min. TW: non-activated tap water (negative control); Control (pH): distilled water pH 3.5. Different superscript letters indicate significant differences among the groups. ($p < 0.05$) based on ANOVA statistical test followed by Kruskal-Wallis and Dunn's post hoc test.

No significant reduction in the *C. albicans* count was detected after contact with PAW or distilled water with pH 3.5 ($p > 0.05$) exposed for 10 or 30 min periods (Figure 9). These results differ from a previous study in which PAW, generated by the exposure of deionized water exposed to air plasma prepared in a DBD reactor for 5 min, showed an inhibitory effect on the suspension of *C. albicans* [60]. The impact of PAW on fungi species has been little investigated so far, and future studies using different parameters should be performed. The differences in the susceptibility of *C. albicans* to PAW, when compared to bacterial species, can be correlated to other cellular components, notably the fungal cell wall that presents several peculiarities [61].
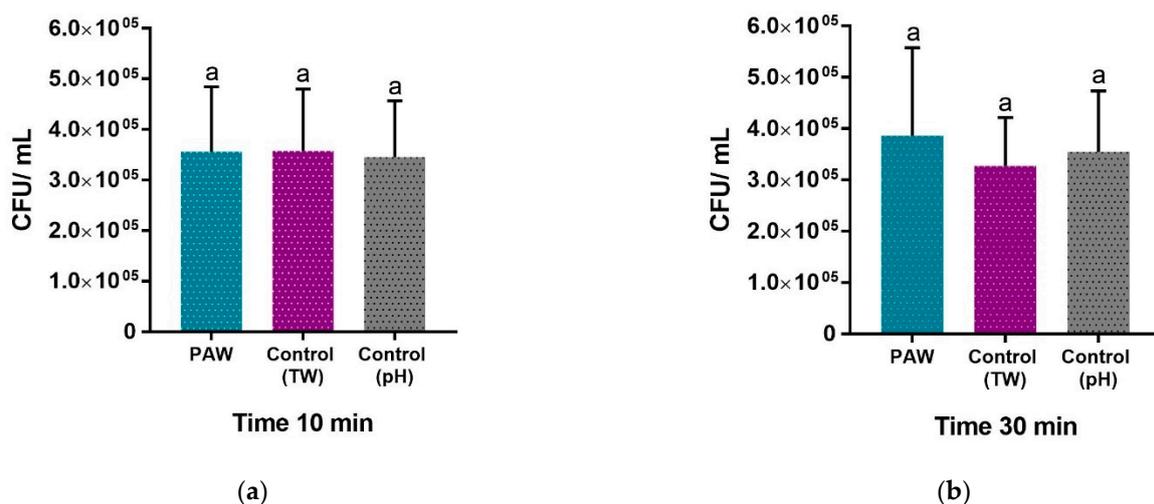
Figure 9. Mean and standard deviation of the number of viable cells of *Candida albicans* after exposure to plasma activated water (PAW) for 10 and 30 min. TW: non-activated tap water (negative control); Different superscript letters indicate significant differences among the groups.($p < 0.05$) based on ANOVA statistical test followed by Tukey's post hoc test.

The antimicrobial effect of PAW has been correlated with reactive oxygen and nitrogen species [62]. Oxidizing species are formed in aqueous solutions and can vary considerably depending on the gas mixture used for plasma formation [63], but the presence of nitrites, nitrates, and H_2O_2 is highlighted [64]. The role of peroxynitrite on the antimicrobial

activity of PAW has been also suggested [30,60] as it can damage biologic molecules, such as membranes and DNA [65]. In the present study, reactive species' presence was proven by a set of parameters such as pH, the potential of oxidizing and reduction, and electrical conductivity [66].

Another factor often related to the antimicrobial effect is acidification. Previous studies have suggested that water acidification and the presence of reactive species (such as OH, NO_{2-}, OH^-) act synergistically in PAW [60,62]. To investigate the extent of pH effect on antimicrobial activity, microbial suspensions were exposed to distilled water adjusted to pH 3.5 with HCl. However, it is essential to note that PAW inhibition and distilled water with pH 3.5 should be compared with caution since HCl is a strong oxidizer.

Interestingly, the bacterial species included in this study behaved differently in these trials. For *S. aureus*, PAW and distilled water at pH 3.5 showed similar inhibitory effects after 10 and 30 min of exposure, suggesting that acidification has an essential role in the inhibitory process. On the other hand, for *E. coli*, PAW was significantly more effective than distilled water at pH 3.5 after 10 min of exposure, suggesting that reactive species played a central role. It can also be inferred that short-lived reactive species mediate the inhibition of *E. coli* in PAW since no inhibitory effect was observed after 30 min of contact. Nitric oxide, hydroxyl radicals, superoxide, peroxynitrite, and peroxynitrite are the short-lived reactive species most frequently encountered [63].

4. Conclusions

This experimental study explored and discussed the antimicrobial effect of plasma activated tap water (PAW) prepared using an atmospheric pressure gliding arc plasma jet generated in a forward vortex flow reactor (FVFR) type. Its potential antimicrobial activity was investigated against the following microorganisms: *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. For this, we carried out a comprehensive characterization of the physicochemical characteristics of PAW in two treatment conditions: stagnant water and agitated water at 200 rpm. The PAW obtained under agitation conditions showed a more uniform acidity between the areas evaluated with pH 3.5 and ORP of 215 mV. These parameters are related to the long-lived RONS species, namely, ozone (O_3), hydrogen peroxide (H_2O_2), nitrates (NO_3^-) and nitrites (NO_2^-) [8,52–55]. Thus, the FVFR G arc system with moving water proved to be a very efficient activation treatment for tap water compared to the DBD system [46] and Inductively Limited Discharge [24]. Finally, *S. aureus*, *E. coli* and *C. albicans* were put in contact with PAW to assess antimicrobial activity through statistical analysis.

According to our results, PAW has an excellent antimicrobial potential to inactivate *S. aureus* and *E. coli* due to the low pH 3.5 and high oxidation-reduction potential (ORP) of 220 mV achieved by the FVFR G arc. This antibacterial effect is related to the interaction between reactive species (RONS) present in PAW and microbial species. The presence of RONS in PAW was confirmed by a set of parameters such as pH, ORP and electrical conductivity [66]. Probably, the interaction between long-lived RONS (H_2O_2, NO_3^- and NO_2^-) and bacterial species was responsible for damaging biological molecules, such as membranes and DNA [65]. For *S. aureus*, the results suggest that there is a dependence between acidification and the inhibitory process due to similar inhibitory effects after 10 and 30 min of exposure to PAW and distilled water at pH 3.5. For *E. coli*, PAW was significantly more effective, showing that reactive species played a central role. However, no significant antimicrobial activity has been achieved for *C. albicans*. Further studies are necessary to improve antifungal activity of PAW produced by gliding arc plasma jet.

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