

OPTIMIZATION AND CHARACTERIZATION OF PLASMA-ACTIVATED WATER FOR BIOMEDICAL DECONTAMINATION APPLICATIONS

Eliza-Maria BALUS¹, Ioana TOFAN², Daniela IONIȚĂ^{3*}, Nicolae GRIGORE³,
Camelia UNGUREANU³

¹ National University of Science and Engineering Politehnica Bucharest, Faculty of Automatic Control and Computer Science, 313 Splaiul Independentei, Sector 6, 060042 Bucharest, Romania, email: eliza_maria.balus@stud.acs.upb.ro (E.M.B.)

² National University of Science and Engineering Politehnica Bucharest, Faculty of Medical Engineering, 313 Splaiul Independentei, Sector 6, 060042 Bucharest, Romania, email: ioana.tofan1409@stud.fim.upb.ro (I.T.)

³ National University of Science and Engineering Politehnica Bucharest, Faculty of Chemical Engineering and Biotechnology, 313 Splaiul Independentei, Sector 6, 060042 Bucharest, Romania, email: daniela.ionita@upb.ro (D.I.); email: nicolae.grigore0712@stud.chimie.upb.ro (N.G.); email: camelia.ungureanu@upb.ro (C.U.)

* Correspondence: daniela.ionita@upb.ro; (D.I.);

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Plasma Activated Water (PAW) is increasingly investigated as an environmentally friendly alternative to conventional chemical disinfectants for biomedical applications. In the present study, a laboratory-scale atmospheric-pressure corona discharge reactor was optimized in order to improve plasma-liquid interaction and enhance the transfer of reactive species into water. The generated PAW was characterized by monitoring pH, electrical conductivity, and hydrogen peroxide concentration, while its antimicrobial activity was evaluated against representative Gram-positive and Gram-negative bacterial strains. The generated PAW also showed antimicrobial activity against all tested bacterial strains. This study shows that optimizing the reactor configuration improves plasma-liquid interaction and enables the reproducible generation of PAW with physicochemical and biological properties suitable for environmentally friendly decontamination and biomedical applications.

Keywords: cold plasma; PAW; RONS; ecological sterilization; medical engineering.

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1. Introduction

The increasing resistance of pathogenic microorganisms to conventional disinfectants has become a major challenge in modern medical sterilization and infection control. Traditional sterilization methods frequently rely on chemical agents that may generate toxic residues, contribute to environmental pollution, and

induce degradation of temperature-sensitive medical materials [1,2]. Cold atmospheric-pressure plasma (CAP) has become an important research topic because of its antimicrobial properties and its potential use in several biomedical applications, including sterilization, wound healing, infection control, and cancer therapy [1,3]. These developments have also contributed to the rapid growth of plasma medicine, an interdisciplinary field that combines plasma physics, chemistry, biology, and medical sciences to better understand the interaction between low-temperature plasmas and biological systems [1]. Among plasma-driven strategies, Plasma Activated Water (PAW) stands out as a highly practical approach. The reactive oxygen and nitrogen species (RONS) generated in the discharge are dissolved into an aqueous medium. This transfer process forms an activated solution containing both short-lived reactive species and more stable compounds, such as hydrogen peroxide, hydroxyl radicals, nitrites, and nitrates [4,5]. The interaction of these reactive species with microbial cells causes oxidative stress, a phenomenon that leads to cell membrane damage, protein oxidation, and nucleic acid degradation, thus explaining the antimicrobial activity characteristic of plasma-activated water [3,6]. The composition and properties of PAW are influenced by both the plasma source and the operating conditions used during treatment. Parameters such as the discharge regime, electrode geometry, treatment time, and the distance between the plasma and the liquid surface affect the transfer of reactive species into the aqueous phase [5,7]. Plasma activation is generally accompanied by a decrease in pH and an increase in electrical conductivity, two parameters that investigators commonly use as indirect indicators of the efficiency of reactive species generation [4,7]. The antimicrobial properties of PAW have been widely documented, the efficiency of reactive species generation still depends strongly on reactor design and operating conditions. Our current work builds upon a previously developed laboratory prototype, whose reactor configuration was further refined to improve plasma-liquid interaction and enhance the transfer of reactive species into water. We subsequently used this optimized system to generate PAW, which was characterized by pH, electrical conductivity, and hydrogen peroxide measurements. Finally, we evaluated its antimicrobial activity against representative Gram-positive and Gram-negative bacterial strains.

The novelty of the present study resides in the optimization of a compact atmospheric-pressure corona discharge reactor specifically designed to improve plasma-liquid interaction and enhance the generation of reactive species in PAW. In addition, an automated prototype capable of reproducible PAW production was developed and experimentally validated. The combined investigation of physicochemical characteristics, storage stability, and antimicrobial performance provides an integrated evaluation of PAW for potential biomedical decontamination applications.

2. Materials and Methods

2.1 Experimental Setup for Plasma Activated Water Generation

The experimental study started with the laboratory prototype shown in Fig. 1. Preliminary tests carried out with this configuration indicated that, although plasma generation was successfully achieved, the stability of the discharge and the transfer of reactive species into the liquid could be further improved. Based on these observations, the reactor geometry was subsequently refined before the physicochemical and antimicrobial investigations presented in this work.

PAW was generated using an atmospheric-pressure corona discharge system designed to promote the transfer of reactive species into the liquid phase. A laboratory prototype was designed and built for the generation of plasma-activated water (PAW). The system is powered by a high-voltage AC power supply operating in the range of 10–20 kV at a discharge frequency of 20–30 kHz.

The prototype developed in this study is shown in Figure 1. It integrates the high-voltage power supply, voltage monitoring unit, pump controller, plasma discharge control system, and plasma reactor cell into a compact experimental platform.

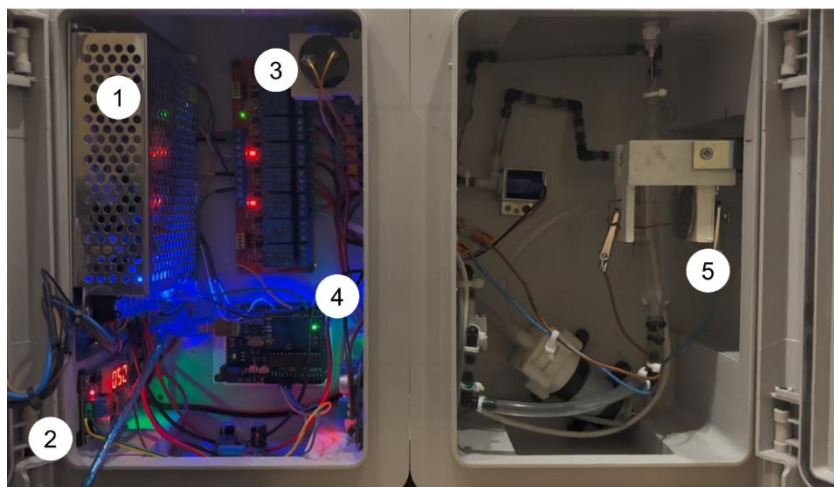


Figure 1. The experimental laboratory prototype developed in this study for the generation of plasma-activated water (PAW): (1) high-voltage alternating current power supply; (2) voltage monitoring unit (voltmeter); (3) pump controller; (4) plasma discharge controller; (5) plasma reactor cell.

Following the preliminary tests, we modified the reactor geometry by using two asymmetrically positioned stainless-steel electrodes. We immersed the grounded electrode directly in the deionized water at the bottom of the reactor vessel, whereas we positioned the high-voltage electrode—designed as a multi-tip

corona structure—approximately 2–3 mm above the liquid surface (Fig. 2). Through this configuration, the plasma discharge occurred directly at the plasma–liquid interface, facilitating the transfer of reactive oxygen and nitrogen species (RONS) into the aqueous phase and intensifying the interaction between plasma and liquid [5]. All experiments were carried out using 150 mL of deionized water maintained at room temperature (22 °C). During plasma treatment, the interaction between the electrical discharge and the liquid gradually modified the physicochemical properties of the solution, leading to a decrease in pH and an increase in electrical conductivity.

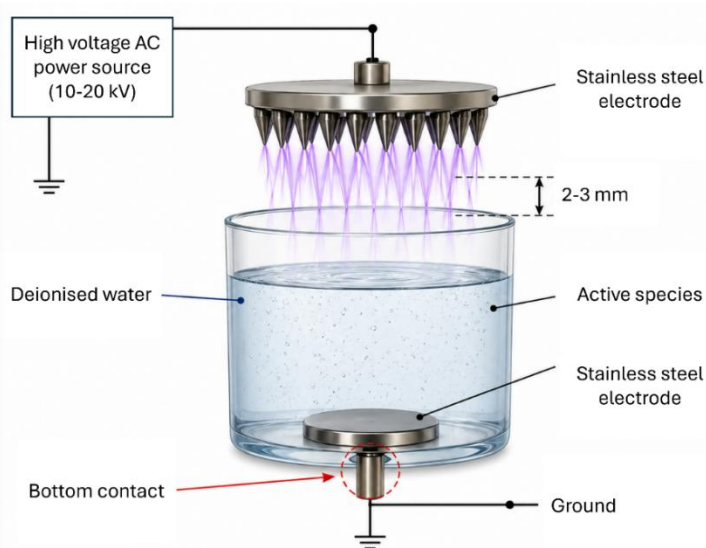


Figure 2. Schematic representation of the optimized reactor used for PAW generation

2.2 Physicochemical Characterization

The physicochemical characterization of Plasma Activated Water (PAW) was performed immediately after plasma treatment to evaluate the main changes induced in the liquid phase. The analysis included pH measurements, electrical conductivity determination, and spectrophotometric quantification of hydrogen peroxide (H_2O_2), which are commonly used to assess the effectiveness of plasma activation. The pH of each PAW sample was measured immediately after plasma activation using a calibrated digital pH meter. These measurements were used to monitor the acidification of the solution associated with the formation of plasma-generated reactive species. Electrical conductivity was determined using a Mettler Toledo Seven Compact S230 conductivity meter. The conductivity values provided an indirect indication of the accumulation of ionic species generated during plasma treatment. Hydrogen peroxide (H_2O_2), one of the main long-lived reactive oxygen species present in PAW, was quantified spectrophotometrically using a JASCO V-

770 UV–VIS–NIR spectrophotometer. The determination of hydrogen peroxide concentration was performed by UV–Vis spectrophotometric measurements at a wavelength of 240 nm, using standard H₂O₂ solutions for calibration, according to the method described in the literature [8]. To obtain the calibration curve, solutions with concentrations ranging from 0 to 100 µM H₂O₂ were prepared, and their absorbances were recorded before performing the sample analyses. The calibration curve thus obtained was used for the quantitative determination of the hydrogen peroxide concentration in plasma-activated water (PAW) samples. In addition, the UV–Vis spectra were recorded in the range corresponding to the characteristic absorption band of H₂O₂ (approximately 240 nm), which allowed the monitoring of its concentration variation during the plasma activation process.

2.3 Antimicrobial activity

The antimicrobial activity of PAW was evaluated against both Gram-negative and Gram-positive bacterial strains, *Escherichia coli* ATCC 11229, *Staphylococcus aureus* ATCC BAA-1026, and *Enterococcus faecalis* ATCC 29212. Microbial suspensions were prepared and adjusted to a 0.5 McFarland standard. The bacterial strains were uniformly spread on the surface of Luria-Bertani agar (LBA) medium plates using the well diffusion method. Sterile wells were subsequently formed in the agar medium, followed by the addition of PAW samples obtained at different plasma activation times (T1, T2, T5, T10, and T15). Distilled water was used as the negative control sample. The inoculated plates were incubated at 37 ± 1 °C for 24 h. Antibacterial effect was assessed by measuring the diameter of the inhibition zones, and the results were expressed in millimeters.

2.4 Statistical Analysis

All measurements were carried out in triplicate, and the data are presented as mean values with standard deviation. One-way ANOVA and Tukey's HSD test were computed to test if the observed differences between treatments were statistically relevant. A significance level of $p < 0.05$ was considered throughout the analysis.

3. Results and Discussion

3.1 Optimization of Plasma–Liquid Interaction

The first experiments were carried out using the initial reactor configuration, in which both electrodes were positioned above the liquid surface. In the initial reactor configuration, the plasma discharge developed predominantly

in the gas phase, limiting the direct transfer of plasma-generated reactive species into the liquid.

This behavior was reflected in the physicochemical properties of the treated water. Only slight changes in pH and electrical conductivity were observed, indicating that a relatively small amount of reactive oxygen and nitrogen species (RONS) reached the aqueous phase. To enhance the interaction between plasma and liquid, the reactor geometry was subsequently redesigned. The ground electrode was immersed directly in the deionized water, while the multi-type high-voltage electrode was positioned approximately 2–3 mm above the liquid surface. Under these operating conditions, the plasma discharge was established directly at the plasma–liquid interface, facilitating the transfer of reactive species into the solution while minimizing their loss to the surrounding atmosphere [5,8,9].

The modified reactor configuration produced a clear improvement in the characteristics of the generated Plasma Activated Water. A faster decrease in pH together with a more pronounced increase in electrical conductivity indicated a more efficient generation and transfer of reactive species into the liquid phase. These findings demonstrate that both the electrode arrangement and the plasma–liquid distance are key parameters governing the efficiency of the PAW generation process.

3.2 Evolution of pH and Electrical Conductivity

The evolution of pH and electrical conductivity during plasma activation was investigated to evaluate the physicochemical modifications induced in the liquid phase. Following optimization of the plasma reactor configuration, a rapid decrease in pH was observed during the first minutes of plasma treatment. The pH decreased from 6.8 to 2.6 after 15 min of activation, reflecting the pronounced acidification of the solution. Similar acidification behavior has been widely reported in atmospheric-pressure plasma-activated water systems, where final pH values between 2 and 4 are commonly obtained depending on discharge power, plasma source configuration, and gas composition [6,7].

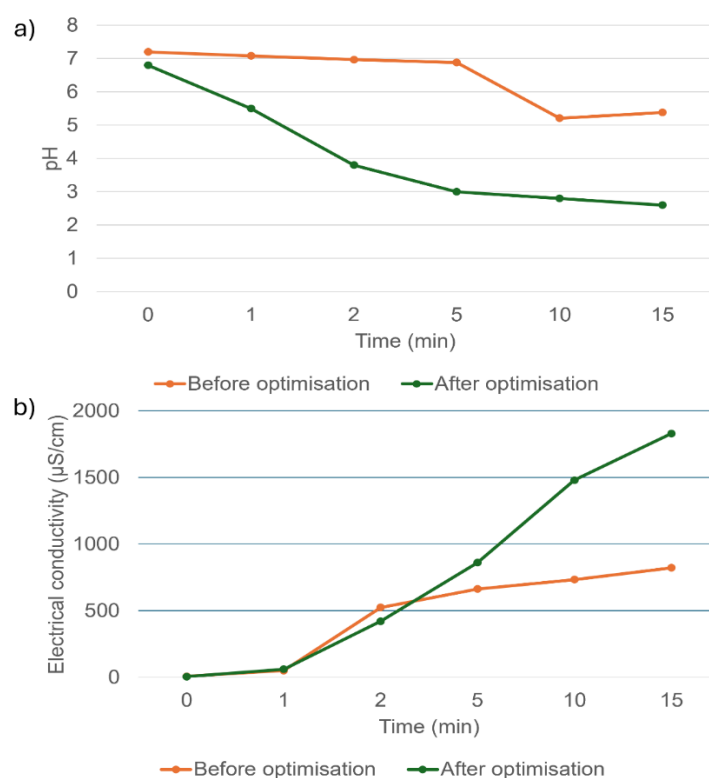
At the same time, electrical conductivity increased from 4.82 $\mu\text{S}/\text{cm}$ to 1830 $\mu\text{S}/\text{cm}$, consistent with the continuous accumulation of ionic species produced during plasma treatment. Previous studies associated conductivity increases in PAW with the formation of nitrates, nitrites, hydrogen peroxide, and other long-lived reactive species [6,9,10]. Table 1 presents the evolution of pH and electrical conductivity values obtained before and after optimization of the plasma activation system.

The evolution of pH and electrical conductivity before and after reactor optimization is illustrated in Figure 3.

Table 1. Evolution of pH and electrical conductivity during plasma activation before and after reactor optimization.

Plasma activation time (min)	Before optimization		After optimization	
	pH	Electrical conductivity ($\mu\text{S}/\text{cm}$)	pH	Electrical conductivity ($\mu\text{S}/\text{cm}$)
0	7.2	7.42	6.8	4.82
1	7.08	50.5	5.5	60.42
2	6.97	524	3.8	420.6
5	6.88	663	3	860.4
10	5.21	733	2.8	1480
15	5.38	822	2.6	1830

The physicochemical changes observed after reactor optimization indicate that the modified configuration enhanced plasma-liquid interaction and favored the transfer of reactive species into the aqueous phase. The simultaneous acidification of the solution and the increase in electrical conductivity are consistent with the characteristic behavior of efficiently activated water.

**Figure 3.** Evolution of (a) pH and (b) electrical conductivity during plasma activation.

3.3 Stability of Plasma-Activated Water Over Time

The physicochemical properties of PAW were also monitored after plasma treatment to evaluate the stability of the activated solution during storage. Particular attention was given to the persistence of long-lived reactive species, which are considered to play a major role in the antimicrobial activity of PAW. For this purpose, pH and electrical conductivity were measured at different time intervals after plasma activation. Changes in these parameters were used as indirect indicators of the persistence of reactive species in the aqueous phase. Table 2 presents the evolution of pH and electrical conductivity during PAW storage following plasma activation.

Table 2. Evolution of pH and electrical conductivity during PAW storage after plasma activation.

Storage time (h)	Electrical conductivity ($\mu\text{S}/\text{cm}$)	pH
1 h	2010	2.5
6 h	2200	2.4

Only minor variations in pH and electrical conductivity were observed after 1 h and 6 h of storage. Since the study considered the short-term stability of plasma-activated water, further investigation into its behavior during storage for longer periods and under different storage conditions is needed. Such studies will contribute to defining the optimal conditions of use and evaluating the practical applicability of PAW in microbiological decontamination processes and in the biomedical field. The relatively constant values of pH and electrical conductivity during the storage period indicate that some of the long-lived reactive species remained present in the aqueous phase. The slight increase in conductivity after 6 h can be explained by secondary reactions taking place in the solution, which favor the formation or additional stabilization of ionic species.

The stability of plasma-activated water (PAW) is influenced by several factors, including storage temperature, exposure to light, composition of the atmosphere in contact with the liquid, and the ratio of short-lived to long-lived reactive species. Short-lived radicals disappear quickly after the plasma treatment is over, while more stable species, such as hydrogen peroxide, nitrites, and nitrates, can remain in solution for longer periods [11,12]. In addition, the reactions that continue during storage can gradually change the chemical composition of PAW, implicitly influencing its biological activity.

The results obtained in the first hours after the plasma treatment show that PAW maintains to an important extent its physico-chemical properties, which allows its use even after the completion of the generation process. This aspect can be an advantage in biomedical decontamination applications, where immediate use of a freshly prepared PAW sample is not always possible.

3.4 Spectrophotometric Determination of Hydrogen Peroxide

Hydrogen peroxide (H_2O_2) was used as one of the main indicators of reactive oxygen species generated during plasma treatment. The concentration of hydrogen peroxide (H_2O_2) in plasma-activated water (PAW) was evaluated by UV–Vis spectrophotometry, based on the characteristic absorption band located around the wavelength of 240 nm. The quantitative determination was performed according to the Beer–Lambert law, which establishes the relationship between absorbance and the concentration of the analyte.

The calibration curve was obtained using standard H_2O_2 solutions with known concentrations. The UV–Vis spectra recorded for these solutions showed a characteristic absorption maximum around 240 nm, and the absorbance increased proportionally with the hydrogen peroxide concentration. The spectra corresponding to the standard solutions used for calibration are shown in Figure 4.

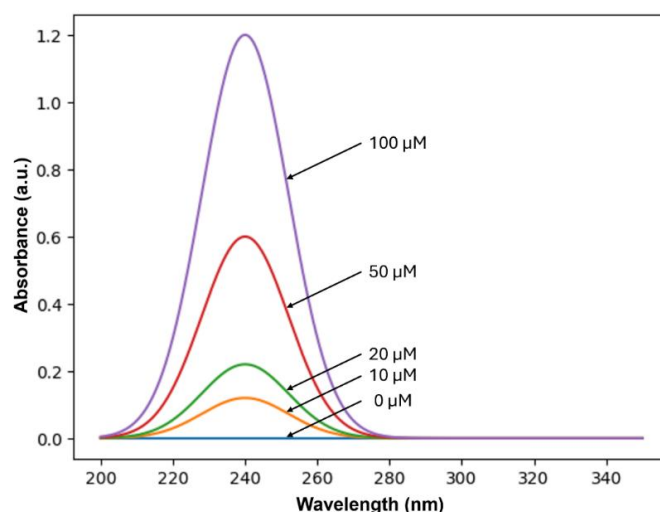


Figure 4. UV–Vis spectra of H_2O_2 calibration solutions.

The calibration curve (Figure 5) was constructed starting from the absorbance values obtained for the standard hydrogen peroxide solutions. In the analyzed concentration range, a linear dependence between absorbance and H_2O_2 concentration was observed, confirming the suitability of the method for the quantitative determination of hydrogen peroxide in the investigated samples.

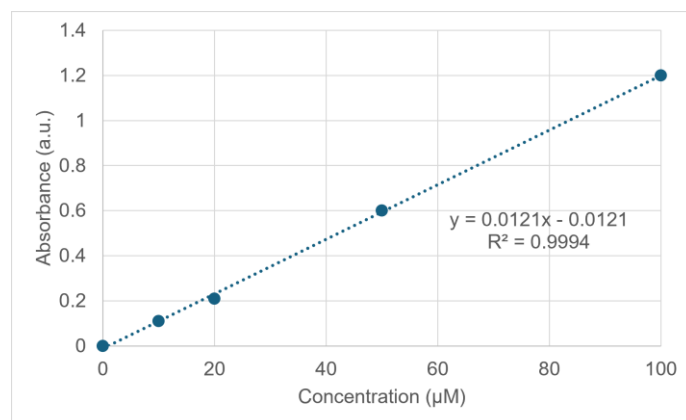


Figure 5. Calibration curve used for spectrophotometric determination of hydrogen peroxide concentration.

The data obtained from the calibration revealed a linear dependence between absorbance (A) and hydrogen peroxide concentration (C), expressed by the following regression equation:

$$A = 0.0121C - 0.0121 \quad (1)$$

where A represents the measured absorbance (u.a.), and C the hydrogen peroxide concentration (μM).

The value of the coefficient of determination ($R^2 = 0.9994$) confirms a very good linear correlation in the analyzed concentration range, indicating that the spectrophotometric method is suitable for the quantitative determination of H_2O_2 . After performing the calibration, UV–Vis spectra were recorded for plasma activated water (PAW) samples obtained at different plasma activation times, the results being presented in Figure 6.

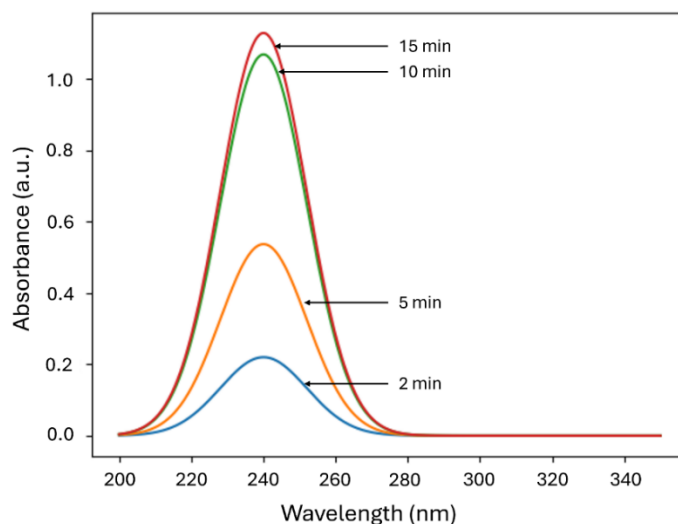


Figure 6. UV–Vis spectra of PAW samples obtained at different activation times.

From the first minutes of plasma activation, an increase in the hydrogen peroxide concentration was observed, indicating the rapid formation of reactive oxygen species in the treated water. The progressive intensification of the characteristic absorption band around 240 nm, with the extension of the activation time, confirms the gradual accumulation of H_2O_2 in the liquid phase. The hydrogen peroxide concentrations were determined based on the calibration curve, and the values obtained for each activation interval are presented in Table 3.

Table 3. Variation of hydrogen peroxide concentration with plasma activation time.

Activation time (min)	H_2O_2 concentration (μM)
1	0
2	20
5	45
10	89
15	94

During the first minutes of plasma activation, a sharp increase in hydrogen peroxide concentration was observed, indicating an efficient generation of reactive oxygen species in the liquid phase. As activation continued, the accumulation of H_2O_2 became slower, suggesting the progressive establishment of an equilibrium between the formation and decomposition processes. Hydrogen peroxide is one of the most stable reactive oxygen species identified in plasma-activated water (PAW). Due to its persistence in solution, it contributes to the maintenance of oxidative effects and is considered one of the factors involved in the antimicrobial activity reported for PAW [3,6]. The concentration values determined in this study are comparable to those reported for other PAW systems operated at atmospheric pressure, where concentrations can vary from several tens to several hundred micromoles, depending on the reactor configuration, treatment duration, discharge power, and working gas composition [4,8].

Correlated with changes in pH and electrical conductivity, these results confirm that plasma treatment produces important physicochemical changes in the aqueous phase and favors the obtaining of plasma-activated water with potential for use in antimicrobial applications.

3.5 Comparative Analysis with Literature

The physicochemical characteristics of the optimized PAW system were analyzed in comparison with the values reported in the literature for plasma-activated water systems operated at atmospheric pressure, in order to frame the experimental results in the context of existing research. Table 4 presents the corresponding literature values together with those measured in the present work for different plasma source configurations.

Table 4. Comparison of physicochemical characteristics with representative atmospheric-pressure PAW systems.

Study	Plasma source	Treatment time	Final pH	Electrical Conductivity	H ₂ O ₂ concentration
[6]	air plasma jet	10 min	~3	significant increase	70–100 μ M
[7]	atmospheric plasma discharge	15 min	2–4	strong increase	50–300 μ M
[4]	various PAW systems	variable	2–5	variable	tens–hundreds μ M
Present work	Corona discharge	15 min	2.6	1830 μ S/cm	94 μ M

The physico-chemical characteristics measured for the optimized PAW system were comparable with those reported for other atmospheric-pressure plasma activation systems. The pronounced decrease in pH together with the increase in electrical conductivity indicates an efficient transfer of reactive species into the aqueous phase. In addition, the hydrogen peroxide concentrations measured in this study fall within the range commonly reported for similar plasma systems. Corona discharge systems are particularly attractive for PAW generation due to their relatively simple reactor design, low operational cost, and efficient plasma generation under atmospheric-pressure conditions [13]. The optimized reactor configuration provided stable PAW generation and produced water with physicochemical characteristics suitable for further biomedical and antimicrobial investigations.

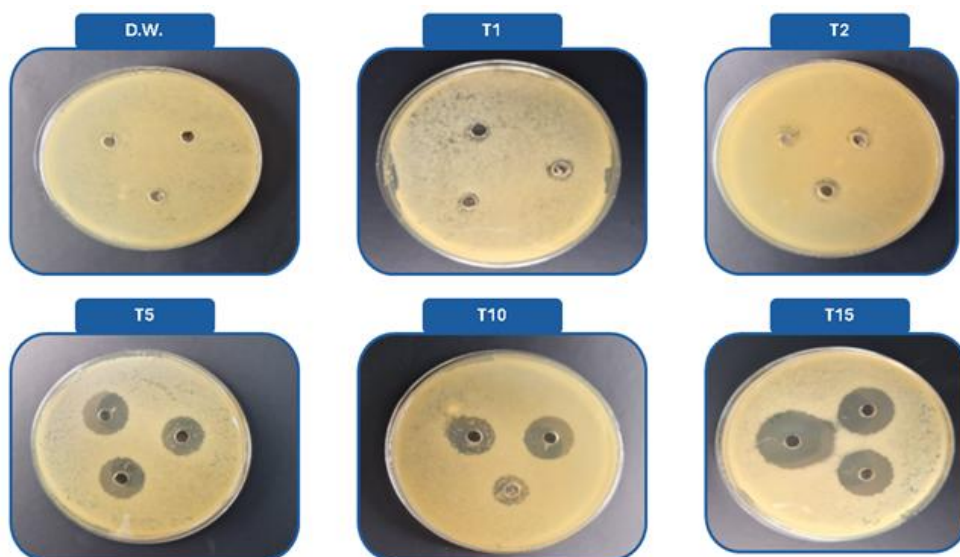
3.6 Antimicrobial Activity of PAW

The antimicrobial properties of Plasma Activated Water have attracted considerable attention in recent years due to the strong oxidative effects induced by plasma-generated reactive species [10,14]. The antimicrobial mechanism of PAW is generally associated with oxidative stress produced by reactive oxygen and nitrogen species, which may induce bacterial membrane disruption, protein oxidation, and nucleic acid damage [3,6, 11]. In the present study, the antimicrobial activity of PAW was evaluated against representative Gram-negative (*Escherichia coli* ATCC 11229) and Gram-positive (*Staphylococcus aureus* ATCC BAA-1026 and *Enterococcus faecalis* ATCC 29212) bacterial strains to assess the biological effectiveness of the generated reactive species. Table 5 presents the inhibition zone diameters (IZD) obtained for PAW samples generated at different plasma activation times, and the sample codes T1 to T15 correspond to the plasma treatment duration, indicating increasing activation times from 1 to 15 min used during PAW generation.

Table 5. Antibacterial activity of PAW evaluated by inhibition zone diameter.

Sample	<i>E. coli</i> , IZD (mm)	<i>S. aureus</i> , IZD (mm)	<i>E. faecalis</i> , IZD (mm)
D.W. (control)	N.D.	N.D.	N.D.
T1	N.D.	N.D.	N.D.
T2	11.0 ± 1.0	7.0 ± 1.0	10.0 ± 1.7
T5	15.0 ± 1.0	18.3 ± 1.5	15.7 ± 1.5
T10	18.3 ± 3.0	17.3 ± 2.9	16.3 ± 4.0
T15	24.0 ± 1.0	25.3 ± 4.2	20.3 ± 4.2

Experimental observations revealed a clear dependence of antimicrobial activity on plasma activation time. No inhibition effect was observed for the control sample and for short activation conditions, whereas progressively larger inhibition zones were obtained for longer plasma treatment durations. The antibacterial activity of PAW against *S. aureus*, *E. coli*, and *E. faecalis* is illustrated in Figures 7–9. The results revealed a gradual increase in antimicrobial activity with increasing PAW treatment time. The largest inhibition zones were observed for T15 samples, suggesting the accumulation of reactive species generated during the plasma process. As possible mechanisms of action, we can consider that plasma water may contain: reactive oxygen species, reactive nitrogen species, hydrogen peroxide, ozone and hydroxyl radicals. These compounds can damage the bacterial cell membrane; induce oxidative stress; may modify cell permeability; and damage proteins and microbial genetic material [15-18].

**Figure 7.** Antimicrobial activity against *Staphylococcus aureus* ATCC BAA-1026 of the tested samples.

Staphylococcus aureus showed the largest zones of inhibition at T15, indicating increased sensitivity to oxidizing compounds generated in water.

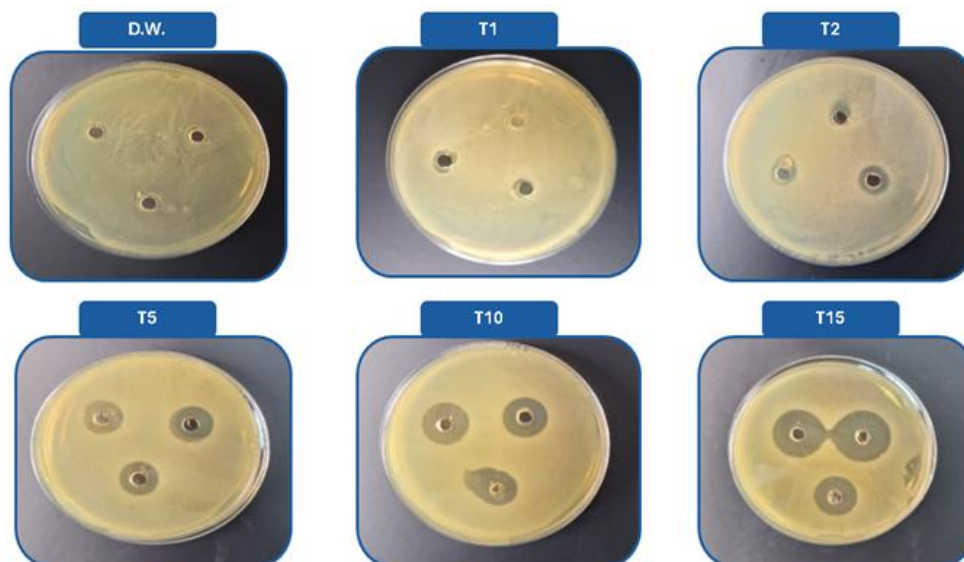


Figure 8. Antimicrobial activity against *Escherichia coli* ATCC 11229 of the tested PAW samples.

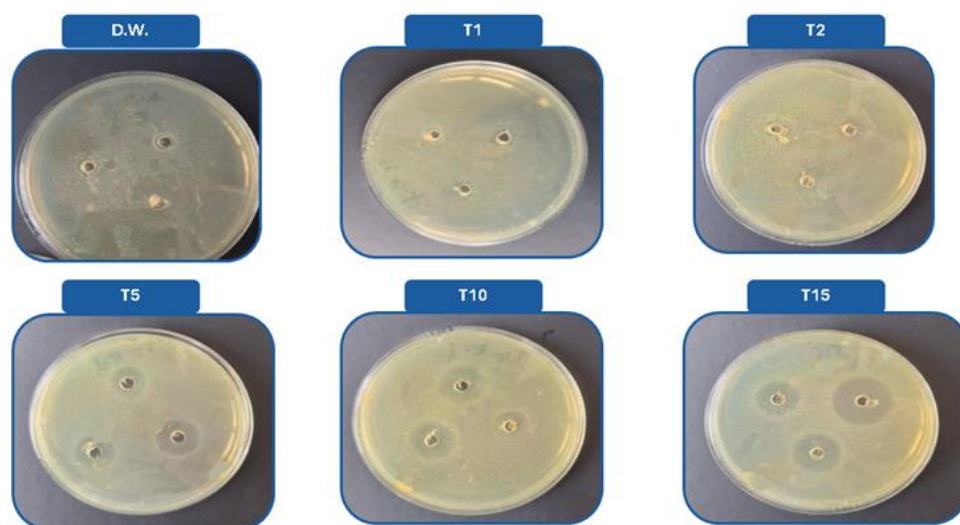


Figure 9. Antimicrobial activity against *Enterococcus faecalis* ATCC 29212 of the tested PAW.

Escherichia coli and *Enterococcus faecalis* also showed significant inhibition, but with variations dependent on the structure of the cell wall and their own antioxidant protection mechanisms.

The statistical analysis indicated significant differences between treatments, especially intermediate to T1 and T15, which showed the highest antibacterial effect.

The results obtained demonstrate that the developed prototype allows the reproducible generation of plasma-activated water, with physicochemical properties and antimicrobial activity that support the potential of this technology for microbiological decontamination applications. Extension of the studies to other microorganisms and conditions of use will allow a more complete evaluation of the implementation possibilities in the biomedical, agricultural, and food safety fields.

4. Conclusions

Optimizing the reactor geometry was an essential element for increasing the efficiency of the plasma-activated water production process. The proximity of the discharge zone to the liquid surface and the modification of the electrode configuration favored the transfer of reactive species to the aqueous phase, an aspect reflected by the more pronounced decrease in pH, the increase in electrical conductivity, and the accumulation of hydrogen peroxide.

The results of the physicochemical characterization show that the developed system reproducibly produces plasma-activated water, with properties comparable to those reported for other plasma-based systems at atmospheric pressure. The stability of the analyzed parameters in the first hours after treatment indicates that the PAW retains sufficient characteristics for use in applications that do not require immediate application.

The antimicrobial activity observed against Gram-positive and Gram-negative bacteria confirms the efficiency of the plasma-activated water generated under the investigated conditions and highlights the contribution of reactive species to the antimicrobial effect obtained.

Overall, the study validates the operation of the experimental prototype and demonstrates its ability to reproducibly generate PAW with appropriate physicochemical and biological properties. The results support the further development of this technology as an alternative for microbiological decontamination processes, with potential applications in the biomedical, food safety and agricultural fields.

Although the obtained results confirm the performance of the proposed system for the generation of plasma-activated water, further investigations are needed to optimize the operating parameters and expand the scope of application. In this sense, future research will analyze the influence of the applied voltage on the formation of reactive species and evaluate the antimicrobial efficiency against fungi, viruses, and microbial biofilms. These studies will allow a deeper understanding of the mechanisms involved and support the development of

microbiological control and decontamination applications based on plasma-activated water.

Author Contributions

Conceptualization, E.M.B, I.T; methodology, E.M.B, I.T and D.I.; validation, E.M.B, I.T and D.I.; formal analysis, E.M.B, I.T, C.U., N.G. and D.I.; investigation, E.M.B, I.T, C.U., N.G. and D.I.; writing—original draft preparation, E.M.B, I.T and D.I.; writing—review and editing, E.M.B, I.T and D.I.; supervision, E.M.B, I.T and D.I. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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Conflicts of Interest

The authors declare no conflict of interest.

Artificial Intelligence Disclosure

Artificial intelligence-based tools were used to improve language clarity, grammar, and readability during manuscript preparation. The tool used was ChatGPT (OpenAI, GPT-5.5). No AI tools were used for study design, data analysis, interpretation of results, or generation of scientific content. The authors take full responsibility for the manuscript.

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