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The effect of low-temperature plasma on microorganisms in water and wastewater

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Abstract: The presence of microorganisms in water and wastewater is of significant importance for public health. Bacteria from the *Enterobacteriaceae* family, such as *E. coli* and *Salmonella sp.*, pose a serious health risk. Their presence in treated wastewater and drinking water is absolutely unacceptable. The aim of the study was to evaluate the effectiveness of the bactericidal properties of low-temperature atmospheric plasma. The research involved treating water and wastewater samples containing suspended bacteria with plasma for varying durations (ranging from 5 seconds to 30 minutes). The plasma stream was generated using a pulsed atmospheric plasma arc, with air as the working gas. The samples contained both microorganisms that naturally occur in wastewater treatment plants and laboratory-cultured strains (in water). The results showed that low-temperature atmospheric plasma effectively eliminates microorganisms, although the required exposure time depends on microbial origin. Laboratory-cultured bacteria were eliminated within 30 seconds of plasma treatment, whereas naturally occurring wastewater microorganisms required up to 20 minutes for effective inactivation. The efficiency of the process depended on many factors such as contact time and microorganism type. In addition to its strong bactericidal and fungicidal properties, low-temperature atmospheric plasma also impacts physicochemical parameters, including pH and electrical conductivity. However, these changes tend to stabilize within 24 hours, particularly in wastewater samples. Overall, cold plasma presents a promising method for water and wastewater disinfection.

Introduction

Wastewater produced in various industrial, domestic and agricultural processes, is a significant source of microbiological threats to the environment and public health. Water that has undergone purification processes but still contains pathogens can lead to serious health problems for humans, animals, and plants (Ezugbe & Rathilal, 2020; Kowalska et al., 2021). One of the main microbiological threats associated with wastewater is the *Enterobacteriaceae* family, including *E. coli* and *Salmonella* species (Jałowicki et al., 2024). These pathogens can cause several serious infectious diseases, such as diarrhea, gastritis, and more serious systemic infections. Due to their high resistance and ability to survive in various environments, their presence in sewage poses a serious health risk (Escobar-Muciño et al., 2022; Yanagimoto et al., 2020). Untreated wastewater that enters the aquatic environment can lead to contamination of groundwater and surface water (Wolska et al., 2024). Contaminated water can then enter water systems, potentially causing widespread outbreaks of waterborne diseases. Even treated wastewater may contain residual pathogens due to deficiencies in the treatment process, posing

additional risks (Lin et al., 2022; Wear et al., 2021). In addition to bacteria, wastewater may also contain viruses, protozoa, and fungi, which can also have negative health effects. Therefore, effective microbiological control and monitoring of sewage are crucial for protecting public health and the environment (Kadadou et al., 2022).

Modern wastewater treatment technologies must therefore include effective methods for eliminating pathogens, along with regular microbiological monitoring, to minimize the risk of their harmful effects on health and the environment (Wypart-Pawul et al., 2023). A promising approach is the use of low-temperature plasma, also known as cold plasma. Cold plasma is an ionized gas composed of a mixture of ions, electrons, radicals, neutral particles, and excited particles (Mouele E.S., et al. 2021). The applications of cold plasma are diverse, ranging from industrial processes and sewage treatment to food sterilization (Fan J., Wu H., Liu R., Meng L., Sun Y., 2021; Nwabor et al., 2022). Its bactericidal properties are influenced by the presence of various electrons, ions, radicals, and UV radiation. Additionally, factors such as the configuration of plasma generation, operating pressure, and the type of discharge gas used play a critical role. These

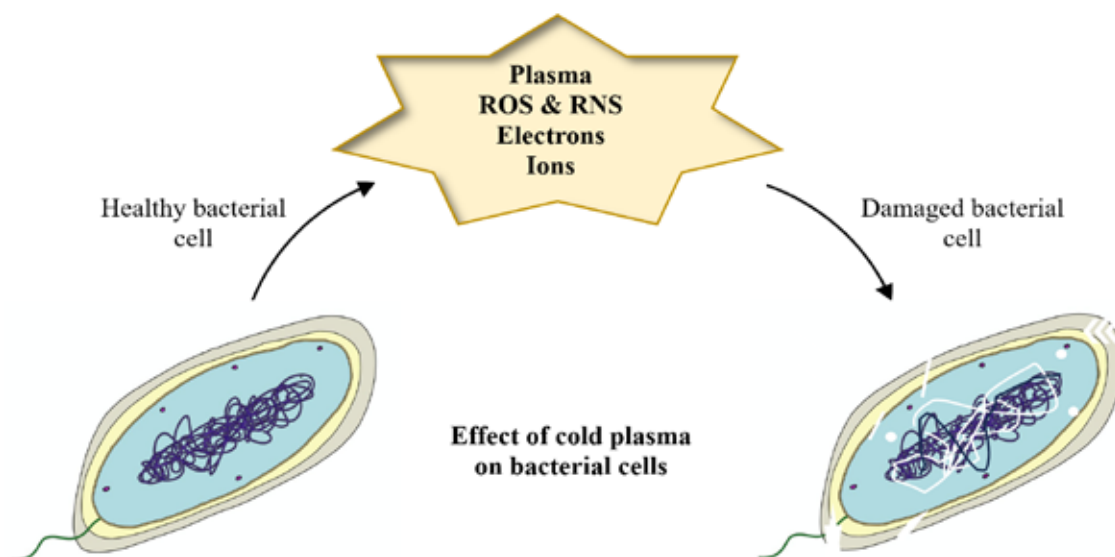


Figure 1. Diagram illustrating the effects of low-temperature plasma on a bacterial cell, developed based on (Mohseni et al., 2023)

components enable the generation of highly reactive oxygen and nitrogen species (RONS) including O , O_2^- , O_3 , OH , OOH , and H_2O_2 ; and NO , NO_2 , NO_3 , N_2O_3 , N_2O_4 , and $ONOO^-$ can be generated (Yahaya et al., 2021).

Reactive components of cold plasma, combined with UV radiation, enhance its antimicrobial properties. Key reactive species involved in bacterial inactivation include ozone, atomic and singlet oxygen, superoxide, peroxide, and hydroxyl radicals (Wypart-Pawul et al., 2024). These molecules disrupt bacterial cell structures, leading to their destruction (Figure 1). The effectiveness of cold plasma (CP) treatment is also influenced by the characteristics of the microorganisms themselves, such as type, strain, growth phase, and initial population size (Mohseni et al., 2023).

Lowering the pH improves the effectiveness of cold plasma (CP) by partially denaturing proteins and causing cell leakage. Water vapor in the gas phase promotes the formation of hydrogen peroxide (H_2O_2), a powerful oxidizer that destroys cells by attacking their outer structures. CP treatment disrupts the structural integrity of microorganisms, ultimately leading to their death. Reactive species generated during CP treatment interact with various microbial components, further enhancing this destructive effect (Mohseni et al., 2023). During plasma discharge, radical species exert antimicrobial effects primarily by inducing oxidative stress, which leads to cellular dysfunction and cell lysis. This stress amplifies damage through membrane poration, enzyme inactivation, lipid peroxidation and DNA fragmentation. Simultaneously, cold plasma species target multiple sites within bacterial and fungal cells, causing structural and functional changes that ultimately result in cell death (Nwabor et al., 2022).

Cold plasma has significant potential in combating microorganisms by generating reactive forms that interact with numerous cellular structures. According to the Regulation of the Minister of Health of 7 December 2017 on the quality of water intended for human consumption, the number of coliform bacteria in drinking water cannot exceed 0 CFU (colony forming units) / 100 ml (Minister of Health of Poland, 2017). The presence of coliform bacteria in water distribution systems may result from factors such as irregularities in water treatment,

ineffective disinfection, secondary contamination, improper cleaning and disinfection after repairs, or the occurrence of reverse flows (Odonkor & Mahami, 2020). Therefore, disinfection of wastewater using low-temperature plasma may be a potential method to eliminate the risk of pathogenic microorganisms entering the natural environment. The aim of the experiment was to evaluate the effectiveness of using low-temperature plasma in eliminating selected microorganisms from wastewater and to determine the optimal conditions for its application. The results of the experiment allowed for an assessment of the potential usefulness of this technology in wastewater treatment and environmental protection.

Materials & Methods

The tests were conducted on two types of medium containing bacteria: (A) distilled water with PBS (phosphate buffer saline) buffer to provide proper osmotic conditions, and (B) treated waste water from a municipal waste water treatment plant using a Membrane Bio Reactor (MBR). Samples of 100 ml of distilled water with PBS (Sample A) were enriched with live cultures of *Escherichia coli* grown under laboratory conditions (24-hour culture, $OD = 1.0$). These samples were then exposed to cold plasma for a time periods ranging from 5 seconds to 5 minutes. Sample B also had a volume of 100 ml and consisted of wastewater treated in the membrane reactor. Samples B were subjected to cold plasma treatment for time intervals ranging from 30 seconds to half an hour.

To generate a cold plasma jet, a CIRRUS plasma generator with a single plasma nozzle (Henniker) was used, as previously described in our previous publication (Wypart-Pawul et al., 2024). This device was used to treat samples from series A. Samples from series B were treated using the Nimbus device (also Henniker), which is equipped with two plasma nozzles. According to the manufacturer, both devices operate under the same parameters, with the primary difference being the number of plasma nozzles.

After plasma treatment of the liquid samples, microbiological cultures were prepared to assess bacterial growth using the plate method. A 0.1 ml aliquot of each sample was inoculated onto

Table 1. Microbiological media used and incubation conditions

Identified pathogen	Microbiological substrate	Incubation temperature	Incubation time	Producer
Escherichia coli	solid, selective Endo medium	36,6°C	24 h	The company BTL sp. z o. o.
Salmonella sp.	Wilson-Blair medium	36,6°C	24 h	The company BTL sp. z o. o.

Petri dishes and incubated according to the guidelines provided by the manufacturer of microbiological media (Table 1).

Additionally, microbiological cultures were prepared from Sample B to determine the effect of cold plasma on the growth of microbiological fungi. The cultures were performed on Sabouraud solid medium with agar (POL-AURA Chemical Reagents), and the Petri dishes were incubated at 26°C for 7 days.

After a specified incubation time, quantitative analysis of microorganisms was performed using the plate method. The procedures performed were in accordance with ISO 4833-1:2013 for *E. coli* and ISO 6579-1:2017 for *Salmonella spp.* The number of bacteria was calculated using the formula:

$$L = \frac{C}{(N_1 + 0,1N_2)} \cdot d \quad (1)$$

where: L - number of microorganisms in 1 cm³ of sample, C - sum of colonies on all plates, N₁ - number of plates from the

first dilution, N₂ - number of plates from the second dilution; d - dilution index corresponding to the lowest dilution counted. No sample dilutions were performed during microbiological cultures.

pH and electrical conductivity (EC) were measured in each of the analyzed samples. The pH was measured using an Elmetron CP-411 series pH meter, and EC was measured using an Elmetron CPC-505 series conductometer. Measurements were taken on the day of plasma treatment and again 24 hours after the tests were conducted.

Results

The quantitative analysis of bacteria was conducted using the plate count method. The tests were carried out to determine the number of colony-forming units (CFU) in the analyzed samples, which allowed for the assessment of the level of presence of microorganisms. Based on the obtained results,

Table 2. Quantitative analysis of bacteria based on the plate method

Sample type	Sample name	Plasma time [min] *seconds	Number of colony forming units [CFU/cm ³]	
			E. coli	Salmonella sp.
A	1.0	control sample	3, 11 · 10 ⁵	-
	2.5	5*	2, 78 · 10 ⁵	-
	3.10	10*	6,20 · 10 ⁴	-
	4.15	15*	4,02 · 10 ³	-
	5.30	≥ 30*	0	-
No bacterial colonies were detected in the remaining Petri dishes				
B	1.0	control sample	4,62 · 10 ⁵	3,48 · 10 ⁵
	2.0,5	30*	4,50 · 10 ⁵	3,39 · 10 ⁵
	3.1	1	4,22 · 10 ⁵	3,29 · 10 ⁵
	4.2,5	2,5	3,79 · 10 ⁵	3,16 · 10 ⁵
	5.5	5	3,42 · 10 ⁵	2,54 · 10 ⁵
	6.7,5	7,5	3,02 · 10 ⁵	2,30 · 10 ⁵
	8.10	10	2,67 · 10 ⁵	3,66 · 10 ⁴
	9.15	15	3,22 · 10 ⁴	4,22 · 10 ³
	10.20	20	1,98 · 10 ³	2,17 · 10 ³
	11.25	≥ 25	0	0
No bacterial colonies were detected in the remaining Petri dishes				

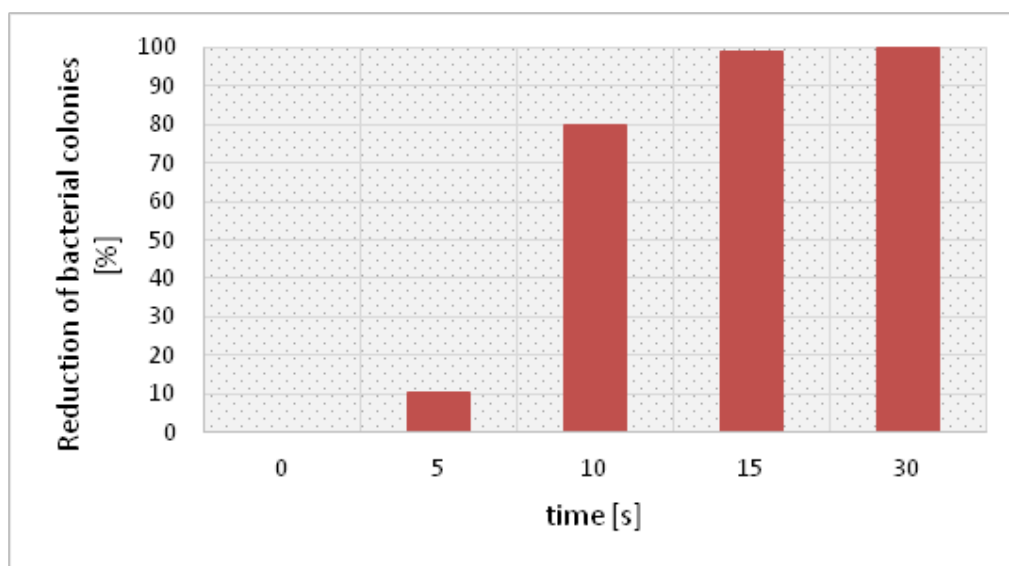


Figure 2. Efficiency of *E. coli* bacteria colony reduction in water under the influence of plasma treatment (samples A)

calculations were performed to estimate the number of bacteria per unit volume of the sample.

Quantitative analysis of *Escherichia coli* and *Salmonella* bacteria

The results of the quantitative analysis performed for *E. coli* (Sample A), *Salmonella* sp. and *E. coli* (Sample B) are presented in Table 2.

Samples containing distilled water with laboratory-grown *E. coli* required significantly shorter plasma exposure times compared to Sample B, which contained wastewater with naturally occurring bacteria. Laboratory-grown *E. coli* strains were found to be more sensitive to cold plasma (Figure 2). In the case of series B samples, the same inhibitory threshold – 25 minutes of plasma exposure – was observed for both *E. coli* and

Salmonella growth (Figure 3). The observed difference in the required exposure time to achieve a bactericidal effect between the samples containing distilled water and those containing wastewater is attributed not only to potential variations in bacterial strain sensitivity but also to the distinct chemical properties of the two matrices. Distilled water presents a clean medium, free from interfering substances, allowing for optimal action of reactive oxygen and nitrogen species generated by cold plasma.

In contrast, wastewater contains numerous organic compounds, inorganic ions, and antioxidant components that can neutralize reactive radicals and species, significantly reducing their availability to interact with microbial cells. This process known as “radical scavenging,” limits the effectiveness of oxidative mechanisms that damage cellular structures. As a

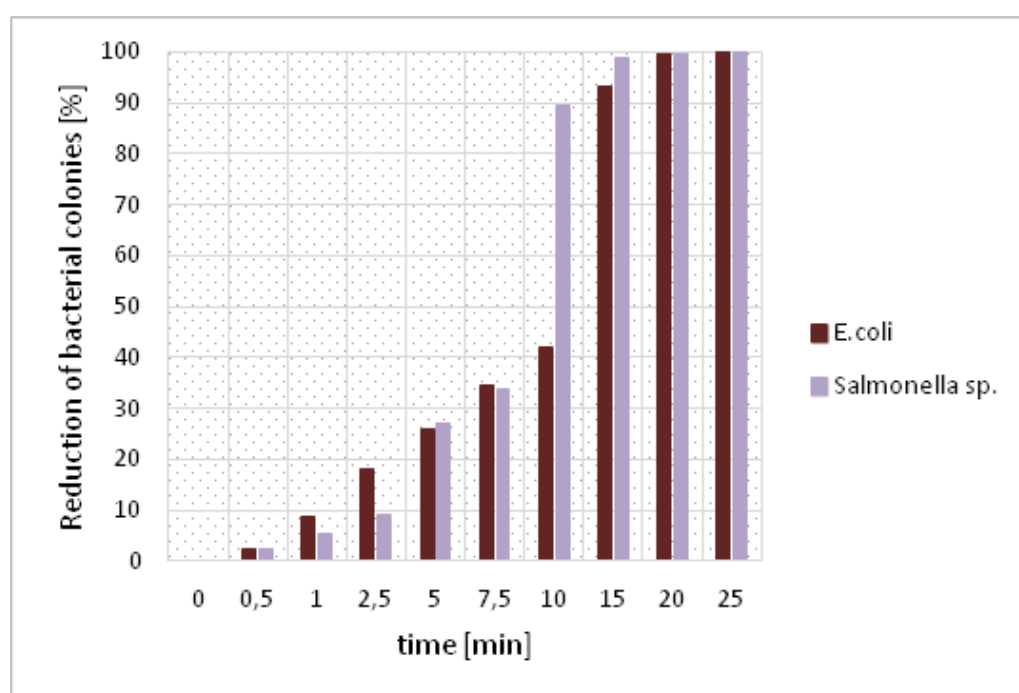


Figure 3. Efficiency of reducing *E. coli* and *Salmonella* sp. bacterial colonies in wastewater under the influence of plasma treatment (samples B)

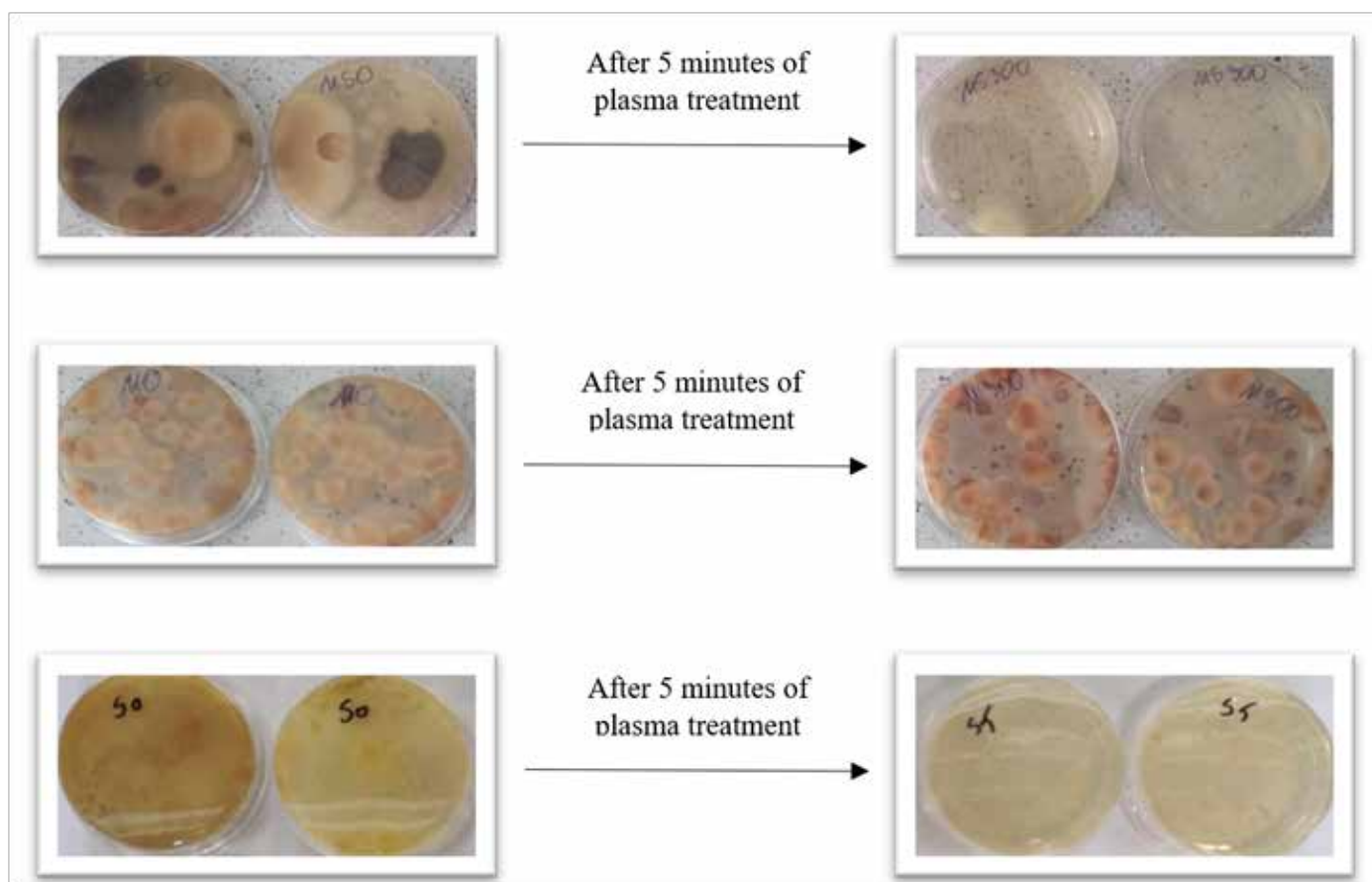


Figure 4. Inhibition of the growth of microscopic fungi as a result of plasma treatment of samples from series B. Effect of 5-minute low-temperature plasma treatment of samples on mycelium morphology

result, the complex chemical composition of wastewater acts as a reactivity buffer, necessitating longer plasma exposure times to achieve comparable bactericidal effects. Nevertheless, the findings confirm the bactericidal efficacy of low-temperature plasma, as shown in Table 3 above.

Evaluation of fungicidal properties

As a result of research into the effect of low-temperature plasma on the growth of microscopic fungi, inhibition of fungal growth due to plasma treatment was found, which is confirmed by Figure 4.

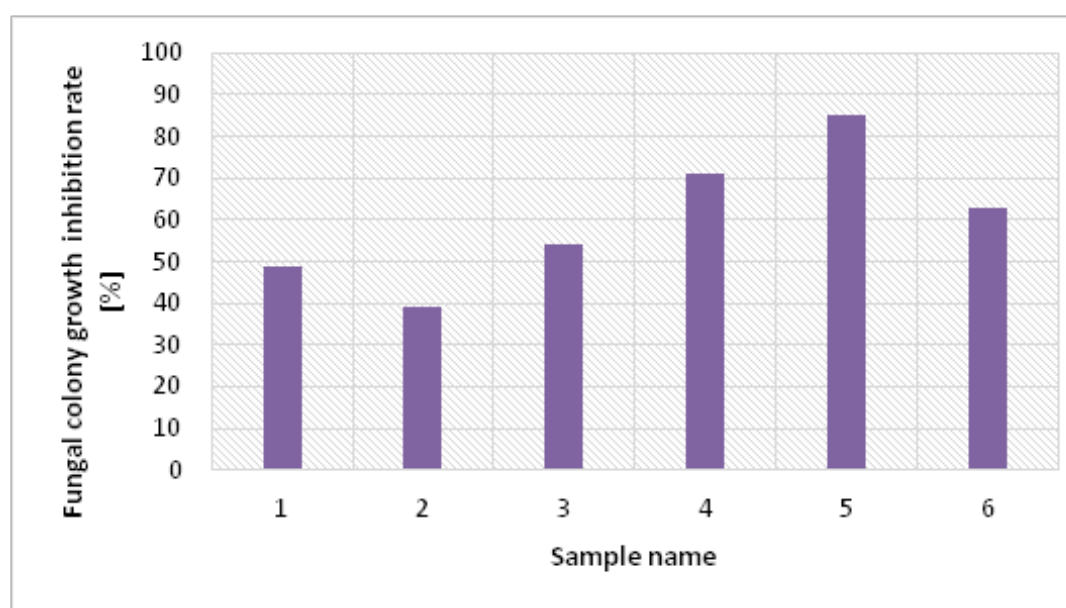


Figure 5. Effect of cold plasma treatment on the number of fungal colonies on Petri dishes (samples B – wastewater). Dishes are labeled 1 to 6, corresponding to individual repetitions. A noticeable reduction in the number of colonies was observed after plasma exposure.

Based on the difference in the number of fungal colonies on the plates (marked with symbols A - F) before and after plasma treatment, a growth inhibition rate of 39–85% was achieved after 5 min of plasma treatment (Figure 5). However, when analyzing Petri dishes with fungal colonies (Figure 2), the most noticeable change is the difference in the morphology of the colonies. Fungal colonies subjected to plasma process are much smaller and lighter in color. The inhibition of the fungal growth under the influence of low-temperature plasma may be related to metabolic disruption or spore damage.

Physicochemical parameters

Monitoring pH and electrical conductivity (EC) provided insight into whether the chemical changes induced by low-temperature plasma contributed to its effectiveness in eliminating *E. coli*, *Salmonella sp.*, and microscopic fungi. Reactive oxygen and nitrogen species (RONS) generated by low-temperature plasma can alter pH potentially impacting microbial survival through acidification or alkalization of the medium. EC measurements

allowed for the assessment of ion concentration in the solution, which may reflect the breakdown of microorganisms, the release of ions from their structures, or chemical changes in the sample after plasma treatment. The changes in these parameters are presented in Figures 6 and 7. The graphs display electrical conductivity (EC) measurements for two types of liquid samples (A and B) subjected to cold plasma treatment, recorded on the day of treatment and 24 hours later.

In Sample A, EC values remained relatively stable, ranging from approximately 500 to 650 $\mu\text{S}/\text{cm}$, with only slight fluctuations during plasma exposure up to 300 seconds. An initial increase in EC observed immediately after treatment suggests the temporary generation of ionic species, likely including H^+ , NO_3^- , or OH^- . However, after 24 hours, a slight decline in EC was noted, indicating partial recombination, neutralization, or degassing of these reactive species. This behavior is consistent with the characteristics of distilled or deionized water, which lacks buffering capacity and contains minimal dissolved solids. In contrast, Sample B exhibited a clear time-dependent increase

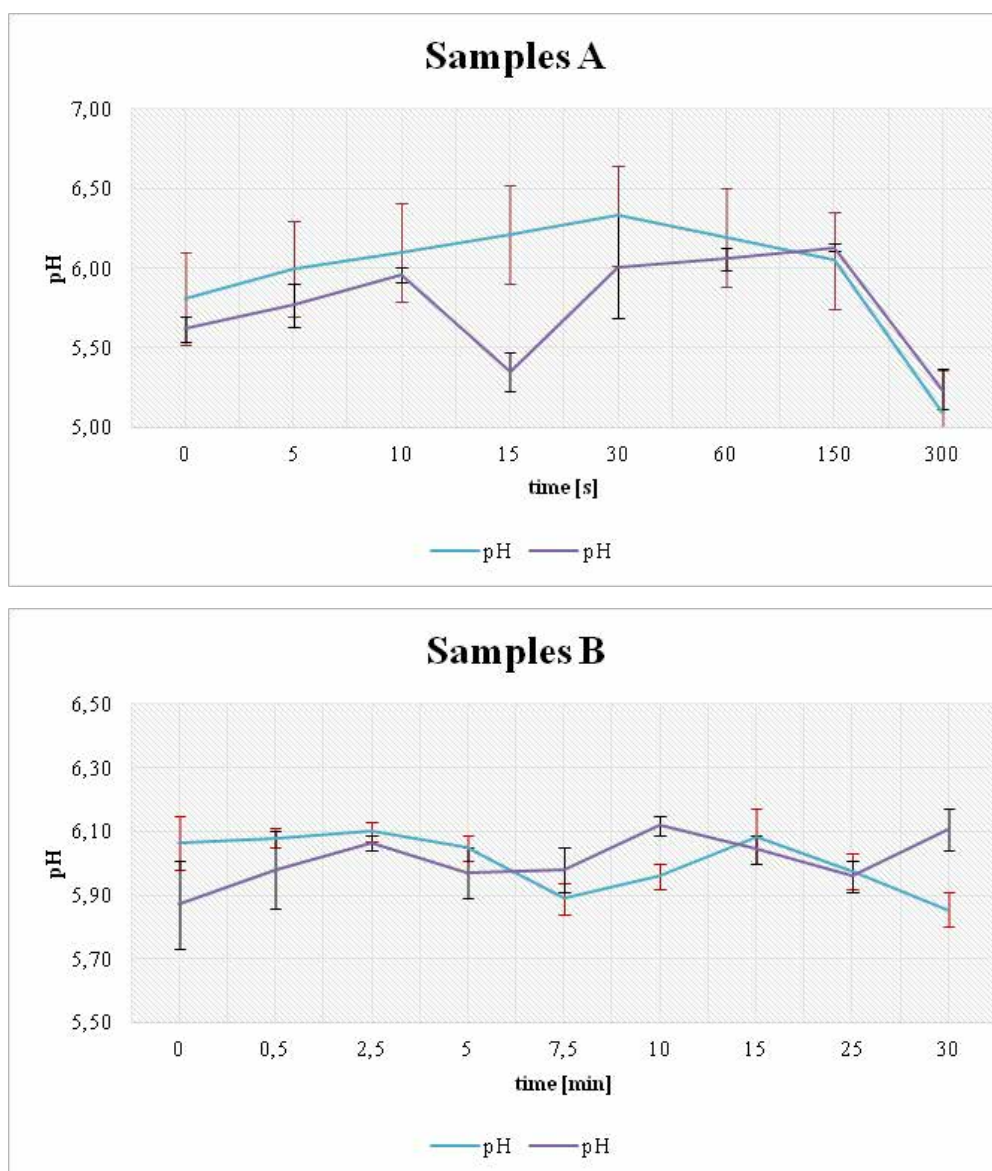


Figure 6. Changes in pH values in samples A and B measured on the day of cold plasma treatment and 24 hours after treatment. The graph presents mean pH values from two replicates, with error bars indicating standard deviation (SD, $n = 2$). Error bars for sample A are marked in red, while those for sample B are shown in black

in EC, rising from around 700 $\mu\text{S}/\text{cm}$ to over 1200 $\mu\text{S}/\text{cm}$ after 30 minutes of plasma exposure. This trend suggests greater production of charged species, possibly due to plasma-induced degradation of organic matter and the formation of nitrate, nitrite, ammonium, and other ions. The higher variability and larger error bars observed in Sample B reflect a more complex matrix, likely a form of treated wastewater containing various buffering agents and reactive compounds. Notably, 24 hours after treatment, EC levels in both samples decreased slightly but remained elevated compared to baseline values. This decline may be attributed to volatilization of reactive nitrogen species, precipitation, or microbial or chemical processes that consume ions. The more pronounced EC increase in Sample B is attributed to its higher ionic strength, greater organic content, and enhanced reactivity under plasma exposure. In contrast, the modest EC shift in Sample A reflects its low conductivity and limited capacity to retain reactive species.

These results highlight the significant influence of matrix composition on plasma-liquid interactions and post-treatment

chemical dynamics. Overall, cold plasma treatment induces measurable changes in liquid conductivity, with stronger effects in complex, buffered systems. The persistence of elevated EC values in Sample B indicates prolonged chemical activity, potentially relevant to disinfection or oxidation processes in wastewater treatment. In sample A, which was treated with low-temperature plasma for 5 minutes, a 15% increase in electrical conductivity was observed compared to the control. This increase may suggest that plasma induces chemical reactions leading to ion release, thereby increasing its conductivity. In sample B, treated with plasma for 30 minutes, the increase in conductivity was as much as 71%, indicating the presence of more reactive dissolved substances in sewage water. The extended treatment time (30 minutes) may lead to more intense reactions, releasing a greater amount of ions, which increase the sample's conductivity. After 24 hours, EC values declined in both samples suggesting a natural stabilization of the chemical environment after plasma treatment.

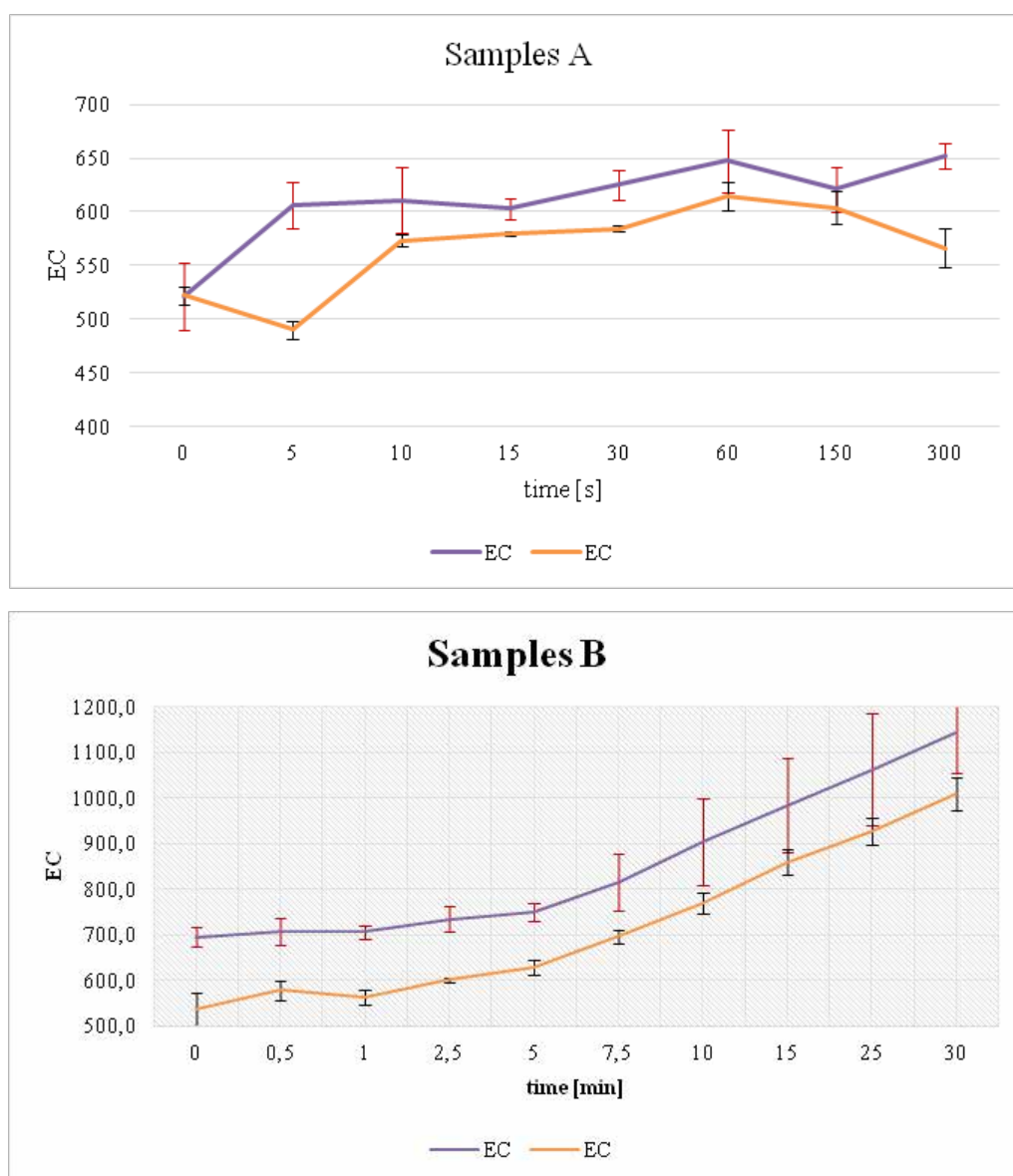


Figure 7. Changes in electrical conductivity (EC) in samples A and B measured on the day of cold plasma treatment and 24 hours after treatment. The graph presents mean EC values from two replicates, with error bars indicating standard deviation (SD, $n = 2$). Error bars for sample A are marked in red, while those for sample B are shown in black

In sample A, treated with low-temperature plasma for 5 minutes, a clear pH decrease from 5.8 to 4.8 was observed, especially with longer cold plasma exposure, indicating the acidifying effect. After 24 hours, the pH remained within a similar range, although a slight decrease persisted at extended treatment durations. This acidification is likely due to the formation of reactive oxygen and nitrogen species that react with water to produce acids such as nitric acid or carbonic acid. Acidification may affect the survival of the studied microorganisms and the lysis process intensification. In sample B, treated with plasma for 30 minutes, the pH decreased from 6.2 to 5.9, which is less pronounced than in Sample A. This suggests that the water in the sewage contains more buffering substances (e.g., organic compounds, ammonia) capable of neutralizing the acidifying effects of plasma. It should be noted that wastewater is naturally alkaline, which may promote the neutralization of acidic reaction products formed during cold plasma treatment. Nevertheless, even a small change in pH indicates that plasma has an impact on the sewage environment. Interestingly, in almost all samples, pH values slightly increased after 24 hours, suggesting a gradual return toward the original pH value, possibly as a result of acid neutralization or reactions with other substances present in the samples. In Sample B (MBR-treated wastewater), pH remained stable between 5.9–6.2 both immediately after treatment and after 24 hours, demonstrating the effectiveness of buffering systems present in the wastewater. The lack of significant pH changes in Sample B indicates high resistance of this matrix to acidifying factors generated by plasma. The observed differences in pH behavior between the two samples primarily reflect variations in buffering capacities and chemical composition of the liquids.

Discussion of results

This section presents a comparison of the obtained results with data available in the literature. The purpose of this comparison is to assess the consistency of the observed effects of cold plasma with the findings of other studies. The study by Klenivskyi et al. (2024) demonstrated effective inactivation of bacteria, including *Escherichia coli*, and microfungi within a few minutes using corona discharge, achieving reductions of up to 10^5 – 10^7 CFU depending on the microorganism. Confirmation of the bactericidal properties of plasma was also presented in the studies by Yahaya et al. (2021). The authors used dielectric barrier discharges (DBD) at atmospheric pressure in an air stream. Research conducted by Xiaoye Lv and Jun-Hu Cheng (2022) aimed to evaluate the effect of cold plasma on *Salmonella typhimurium*. Exposure of the bacteria to low-temperature plasma resulted in disruption of the cell membrane, ultimately leading to cell death. An interesting approach is the use of cold plasma for the inactivation of bacterial spores, as presented in the work of Zhu et al. (2022). This review paper describes the inactivation of spores, including *Bacillus*, in food sterilization. The authors emphasize the need for further research on the mechanism underlying spore inactivation process. They indicate that it is necessary to investigate the effects of cold plasma in combination with other technologies on spore inactivation.

The study conducted by Wei et al. (2024) confirmed the effectiveness of low-temperature plasma in inactivating

Aspergillus niger spores, thereby demonstrating its fungicidal properties. The highest efficiency of the process was achieved after 15 minutes of plasma treatment at 70 kV. Plasma treatment led to the disruption of cell membrane integrity, resulting in the release of intracellular contents. Similar studies have been described in the publication by Ma and Jiao (2022), which focused on the inactivation of fungi and mycotoxins using cold plasma. As in our study, the integrity of the cell membrane was disrupted, resulting in damage to intercellular components and impairing the normal physicochemical functions of the cells.

E. coli and *Salmonella sp.* are particularly sensitive to changes in osmolarity, as disruption of their ionic balance can lead to cell death. An increase in EC may indicate that plasma effectively damages bacterial cells, releasing intercellular ions that contribute to their elimination. Regarding pH changes, the acidification observed after plasma treatment (e.g., from 5.8 to 4.8 in sample A) may directly impact the survival of these bacteria. During cold plasma treatment, reactive oxygen and nitrogen species (ROS and RNS) are generated in water, leading to the formation of nitric, nitrous, and carbonic acids. Since distilled water lacks buffering compounds, even small amounts of these acids can cause a rapid drop in pH.

The EC measurement allowed for the assessment of ion concentration in the solution, which may indicate the breakdown of microorganisms, the release of ions from their structures, or changes in the chemical composition of the sample after plasma treatment. In contrast, treated wastewater after membrane bioreactor (MBR) treatment contains residual salts, ammonia, and organic compounds that contribute to alkalinity – its capacity to neutralize acids. This buffering capacity mainly comes from bicarbonate (HCO_3^-), carbonate (CO_3^{2-}), and hydroxide (OH^-) ions. Although ions such as NH_4^+ , PO_4^{3-} may also be present, their contribution to alkalinity is indirect or minimal compared to the main buffering species. As a result, the pH of wastewater decreases more slowly because the acids generated during plasma treatment are partially neutralized. Moreover, organic matter in the wastewater can react with ROS/RNS, further reducing their acidifying effect. In contrast, distilled water, which has low ionic strength and no buffering capacity, shows a more pronounced pH drop with the same plasma exposure. In wastewater, buffering and alkalinity act as a “shield” against pH decline. Therefore, the faster pH decline observed in distilled water compared to MBR-treated wastewater can be attributed to the absence of buffering compounds. Additionally, the destruction of microorganisms by cold plasma may contribute to the acidification of the liquid through the release of cellular degradation products. However, the primary factor affecting pH reduction is the generation of acids via plasma interactions with water and air – an effect that is more pronounced in weakly buffered solutions.

Following cold plasma treatment, a rapid decrease in pH is typically observed due to the formation of reactive oxygen and nitrogen species (ROS and RNS), that generate acidic compounds such as nitric, nitrous, and carbonic acids. However, after approximately 24 hours, a slight increase in pH is recorded. This delayed pH shift can be attributed primarily to the decomposition of unstable acids, particularly nitrous acid (HNO_2) and carbonic acid (H_2CO_3), which break down into gaseous products like NO_x and CO_2 . The degassing of CO_2

from the liquid phase reduces the concentration of hydrogen ions, thus leading to a minor pH rise. Additionally, residual buffering compounds (e.g., bicarbonates, ammonium, and phosphates) may gradually neutralize the remaining acidity. In treated wastewater, organic matter and microbial residues can also contribute to pH stabilization through sorption or the slow degradation of acidifying agents. Once plasma exposure ceases, the continuous formation of acids stops, allowing equilibrium reactions to gradually shift toward a less acidic state. Moreover, volatile reactive species such as ozone and nitrogen oxides may evaporate from the liquid, eliminating their acidifying potential. In some cases, limited microbial regeneration or enzymatic activity may further metabolize low molecular weight acids. Collectively, these physicochemical and biochemical processes explain the observed slight increase in pH 24 hours post-treatment.

The obtained results indicate that 24 hours after cold plasma treatment, electrical conductivity (EC) slightly increases, while pH remains relatively stable. This phenomenon can be explained by the distinct mechanisms governing each parameter. EC reflects the total concentration of all ions present in the solution, whereas pH refers exclusively to the activity of hydrogen ions (H^+). After plasma exposure, secondary processes such as the release of ions from lysed cells or the breakdown of residual organic matter may occur, leading to increased EC without significantly affecting the acid-base balance. Ions such as Na^+ , K^+ , Cl^- , or SO_4^{2-} contribute to electrical conductivity but do not notably influence the pH. Additionally, the presence of buffering agents in the liquid can effectively compensate for minor fluctuations in H^+ concentration, thereby maintaining pH at a relatively constant level.

Moreover, a lower pH can interfere with bacterial metabolism and cellular functions, further promoting bacterial death. *E. coli* and *Salmonella* are known to be less resistant to acidic environments, so the pH drop caused by plasma treatment may significantly contribute to their elimination. In the case of fungi, changes in conductivity and pH also indicate damage to their cellular structures (e.g., cell walls), leading to ion release and cellular degradation. Although fungi are generally less sensitive to pH changes than bacteria, prolonged exposure to plasma and the resulting acidification can weaken fungal cells, impairing their growth and reproduction. Additionally, plasma-generated reactive species, along with pH changes, may disrupt the overall cellular environment of fungi, further reducing their viability.

Conclusion

This study confirms that low-temperature atmospheric plasma (cold plasma) exhibits strong bactericidal and fungicidal properties, making it a promising tool for water and wastewater disinfection. The results demonstrated that plasma treatment was significantly more effective in eliminating laboratory-cultured bacteria than naturally occurring microorganisms in wastewater, likely due to the protective effects of the complex matrix and buffering components present in treated wastewater. Exposure time played a crucial role, with complete bacterial inactivation observed after 30 seconds in distilled water, and after up to 25 minutes in MBR-treated wastewater. Cold plasma not only disrupts cellular structures through oxidative stress

caused by ROS and RNS, but also influences physicochemical parameters, such as pH and EC, which may further enhance its antimicrobial activity.

A noticeable decrease in pH following plasma treatment suggests acidification of the medium, which is more pronounced in distilled water due to its lack of buffering capacity, while wastewater matrices demonstrated more stability due to their inherent alkalinity. Changes in EC indicated ion release from lysed cells and chemical reactions induced by plasma exposure, with a more pronounced increase observed in wastewater samples. The observed post-treatment stabilization of pH and the slight increase in EC after 24 hours suggest ongoing chemical and biological processes, including acid neutralization, gas release, and buffering effects. Plasma exposure also significantly reduced fungal growth and altered colony morphology, indicating structural damage and disruption of fungal metabolic functions.

Overall, cold plasma is a non-thermal, efficient, and environmentally friendly disinfection method that can be adapted for use in different types of water matrices. However, its effectiveness depends on several factors, including treatment duration, microorganism type, and chemical composition of the treated medium. Further research is recommended to optimize operational conditions and to evaluate long-term effects, especially in full-scale wastewater treatment systems.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Wpływ plazmy niskotemperaturowej na mikroorganizmy w wodzie i ściekach

Obecność mikroorganizmów w wodzie i ściekach ma istotne znaczenie dla zdrowia organizmów żywych. Bakterie z rodziny Enterobacteriaceae, takie jak *E. coli* i *Salmonella* sp., stanowią poważne zagrożenie dla zdrowia. Ich obecność w oczyszczonych ściekach i wodzie pitnej jest absolutnie niedopuszczalna. Celem badania była ocena skuteczności właściwości bakteriobójczych niskotemperaturowej plazmy atmosferycznej. Badaniami objęto próbki ścieków i wody z zawieszonymi bakteriami, które poddano obróbce plazmowej przez różny czas (od 5 sekund do 30 minut). Strumień plazmy generowano za pomocą pulsacyjnego łuku plazmowego w atmosferze, przy czym gazem roboczym było powietrze. Próbkę zawierały zarówno mikroorganizmy występujące naturalnie w oczyszczalniach ścieków, jak i szczepy hodowane w warunkach laboratoryjnych (testy na wodzie). Wyniki wykazały, że niskotemperaturowa plazma atmosferyczna skutecznie eliminuje mikroorganizmy, chociaż czas potrzebny na ich eliminację zależy od ich pochodzenia. Bakterie hodowane w warunkach laboratoryjnych są eliminowane w ciągu 30 sekund od obróbki plazmą, podczas gdy mikroorganizmy naturalnie występujące w ściekach wymagają nawet 20 minut. Skuteczność procesu zależy od wielu czynników, takich jak czas kontaktu lub rodzaj mikroorganizmu. Niskotemperaturowa plazma atmosferyczna wykazuje silne właściwości bakteriobójcze i grzybobójcze, co czyni ją obiecującą metodą dezynfekcji wody i ścieków. Zimna plazma wpływa również na parametry fizykochemiczne, takie jak pH i przewodnictwo elektryczne; jednak zmiany te mają tendencję do stabilizacji w ciągu 24 godzin, szczególnie w ściekach.