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Optimization of bacterial disinfection in drinking water by ozone-induced oxidation-reduction potential control using response surface methodology

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ABSTRACT

This study aimed to determine the optimal oxidation-reduction potential (ORP) and ozonation time to effectively eliminate *Escherichia coli* and *Total coliform* from drinking water. An automated ORP control system was implemented, incorporating a Siemens S7-1500 PLC with HMI interface, ORP and pH sensors, a corona discharge ozone generator, and an electric pump (220 V AC, 60 Hz, 30 W) to maintain continuous water circulation at a flow rate of 20 L/min. A response surface methodology (RSM) experimental design with 13 treatments was used to evaluate ORP levels ranging from 550 to 901 mV and ozonation times from 5 to 210 seconds. Tests were conducted in water with a pH of 6.92, artificially contaminated with *E. coli* O157:H7 and *Total coliform* colonies. The results indicated that ozonation time was the most critical factor in bacterial inactivation, with significant reductions observed beyond 105 seconds at ORP values of 725 mV or higher, achieving complete inactivation of both *E. coli* and *Total coliform*. ANOVA revealed that time and its quadratic term had a statistically significant effect, although the lack of model fit suggests that additional variables may influence the disinfection process. Optimization using Design-Expert software identified 719 mV and 110 seconds as the ideal ORP and ozonation time, respectively, achieving 0 MPN/100 ml for both bacterial groups. These findings provide a robust technical foundation for enhancing water purification systems using automated ozone-based technologies, contributing to safer, more efficient, and sustainable solutions for microbiological treatment of drinking water.

Keywords: automatic control, ozonation, contaminating microorganisms, water treatment.

INTRODUCTION

Universal access to safe drinking water free of pathogens remains one of the main health challenges of the 21st century (Okafor et al., 2024). According to the World Health Organization, the number of people using drinking water sources contaminated by microorganisms present in water is increasing (Shah et al., 2023), posing a risk to drinking water safety, particularly in developing countries. In this context, bacterial contamination of water intended for human consumption constitutes a threat to public health (Ali et al., 2025; Aram et al., 2021), with *Escherichia coli* (*E. coli*) and *Total coliform* being two of the most relevant indicator microorganisms in the evaluation of the microbiological quality of drinking water (Kincaid et al., 2022).

E. coli, a bacterium belonging to the coliform group, is used as a reference parameter to determine the presence of recent fecal contamination,

while *Total coliform* is a group of bacteria found in the environment, including soil, water, and the intestinal tract of humans and warm-blooded animals; its presence in drinking water is used as an indicator of microbiological contamination (Hardjanti et al., 2024). The detection of *Total coliform* in drinking water indicates failures in the treatment system or contamination in the distribution network. Several studies have reported epidemic outbreaks linked to the presence of these bacteria in untreated or poorly disinfected water systems (Lamichhane et al., 2024; Vélez-Reyes et al., 2025).

The disinfection process is one of the most critical stages in drinking water treatment, and its effectiveness depends on both the type of disinfectant and the environmental conditions of use (Wang et al., 2025). In recent decades, ozone (O_3) has become more widely used as a disinfectant agent due to its high oxidizing power and its ability to effectively inactivate bacteria, viruses, and protozoa (Lei et al., 2021; Muzafarov et al., 2021; Rusdiyanto et al., 2023). Unlike chlorine, ozone does not generate potentially carcinogenic organochlorine by-products such as trihalomethanes (THMs) or haloacetic acids (HAAs), which represents a significant advantage in terms of health and environmental safety (Simpson and Mitch, 2022; Xu et al., 2022). It is also possible to use ozone in combination with other oxidizing agents to increase its effectiveness against toxic elements (Zawadzki, 2025).

Ozone acts directly on the cell wall and nucleic acids of microorganisms, breaking down structures essential for their survival (Pérez et al., 2015). This action has proven to be particularly effective against *E. coli* and *Total coliform*, achieving their inactivation in relatively short times and with lower doses compared to other traditional disinfectants (Roobab et al., 2023). However, its high reactivity poses technical challenges related to its dosage, control, and stability in the system. Inadequate treatment, whether due to overdosing or insufficient application, can lead to adverse effects such as the formation of oxidative by-products, material deterioration, or incomplete disinfection that leaves viable bacterial remnants in the water (Golfinopoulos et al., 2024).

Given this problem, monitoring the oxidation-reduction potential (ORP) is a key parameter for evaluating the effectiveness of the ozonation process in real time. ORP reflects the oxidizing capacity of the water being treated, and values above 650 mV have been shown to correlate with a high inactivation rate of *E. coli* and *Total coliform* (Nghi et al., 2018; Wang et al., 2020). Thus, the use of ORP sensors allows for dynamic adjustment of ozone dosing and ensures that an optimal level of oxidation is maintained, contributing to efficient and safe operation of the disinfection system.

However, determining the ideal ORP range for the specific elimination of *E. coli* and *Total coliform* requires the proper interaction between the multiple variables involved, such as ozone concentration, contact time, pH, temperature, and dissolved oxygen demand in the water. It is at this point that Response Surface Methodology emerges as an appropriate statistical tool for the optimization of complex and multifactorial processes. This methodology allows for the quantitative modeling of the relationship between the independent variables of the system and the expected responses, such as bacterial reduction and ORP levels achieved, generating mathematical models that guide the process toward optimal conditions (Feng et al., 2021; Sai Datri et al., 2023).

Several studies have used RSM to optimize disinfection processes in water treatment, demonstrating its effectiveness in minimizing the number of experimental trials required, reducing operating costs, and improving system control. Ezzat and Moustafa (2024), for example, applied this methodology to determine the efficient removal of *E. coli* from wastewater using a new phytomaterial nanozinc with antibacterial potential. Wang et al. (2022) used RSM to evaluate the removal of phenanthrene from soil washing effluent by adsorption with activated carbon. Zhang et al. (2023) used RSM to characterize and optimize the removal of chloride from wastewater using bismuth trioxide. Similarly, Phan et al. (2024) used the response surface methodology to determine the improvement of pharmaceutical wastewater treatment by ozone-assisted electro-oxidation and optimization of accuracy. Ditta et al. (2023) used the response surface methodology to optimize the operating variables of electrochemical water disinfection, where RSM analysis shows that electrode spacing is the most important factor affecting disinfection performance, and increasing electrode spacing inversely affects disinfection efficiency.

Within this framework, the present study aims to optimize the bacterial disinfection process in drinking water by controlling ozone-induced ORP using an algorithm implemented in

a programmable logic controller (PLC), focused on the elimination of *E. coli* and *Total coliform*, using the response surface methodology. Taking into account that there is a range of operational conditions, determined by the interaction between the ozone dose represented by ORP and the contact time, which maximizes the efficiency of removal of these bacteria.

MATERIALS AND METHODS

Process controller

The experimental system was implemented in a drinking water disinfection pilot plant equipped with an ozonation system automatically controlling ORP and ozonation time. The plant integrates a Siemens S7-1500 PLC (model CPU 1516-3 PN/DP) as the main control unit (Jia et al., 2023), and a Siemens TP700 Comfort HMI interface for visualization and monitoring of critical parameters, as shown in Figure 1. The system architecture is based on an Industrial Ethernet network (Profinet). The PLC had a network interface configured with IP address 192.168.0.1 (communication channel with the HMI). The TP700 Comfort HMI was configured with IP address 192.168.0.2 and communicates directly with the PLC through the Profinet port identified as PN/IE_1. This topology allows real-time monitoring of process variables, including ORP reading and control.

Ozone generator

The ozone generator used was of the corona discharge type (Abdykadyrov et al., 2023; Mekkioui and Medjahdi, 2020) depicted in Figure 2, with nominal capacity of 600 mg/h ozone, operates by applying alternating high voltage power of approximately 20 kV in a gas reactor, in which a controlled ionization of oxygen is produced through the corona effect. The system is powered from a conventional alternating current source, which is first converted to direct current by a rectifier stage (AC/DC) and then converted back to high voltage alternating current by a DC/AC inverter. This high voltage signal is applied to a set of electrodes separated by a dielectric, forming an electric field sufficiently intense to induce coronal discharges. Simultaneously, a supply of air or oxygen, provided by a pump, is introduced into the reactor.

In the discharge space, oxygen molecules (O_2) are dissociated into individual atoms due to the energy of the electric field, allowing their subsequent recombination into ozone molecules (Mekkioui and Medjahdi, 2020). This ozonated gas is conducted to the outlet of the system for use in disinfection applications, air treatment, in this specific case in bacterial disinfection of water. Ozone is produced from three oxygen molecules that when bombarded by free electrons are converted into two ozone molecules (Kogelschatz et al., 1988), the chemical reaction of this process is presented in Equation 1.

$$3O_2 + e^- \rightarrow 2O_3 \quad (1)$$

Venturi injector

The Venturi tube was used as a suction ozone injector in water (Figure 3). This process is based on mass continuity and Bernoulli's principle.

The law of conservation of mass states that in a stationary flow all the flow that enters through one side of a circuit must leave through another, this implies that if we decrease the section of the circuit must increase the fluid velocity (Qin and Duan, 2017), it is represented in Equation 2.

$$v_1 A_1 = v_2 A_2 \quad (2)$$

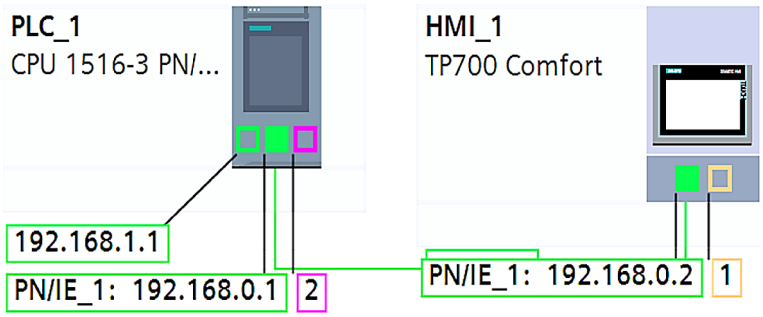

Figure 1. PN/IE network architecture of the ozonation process controller

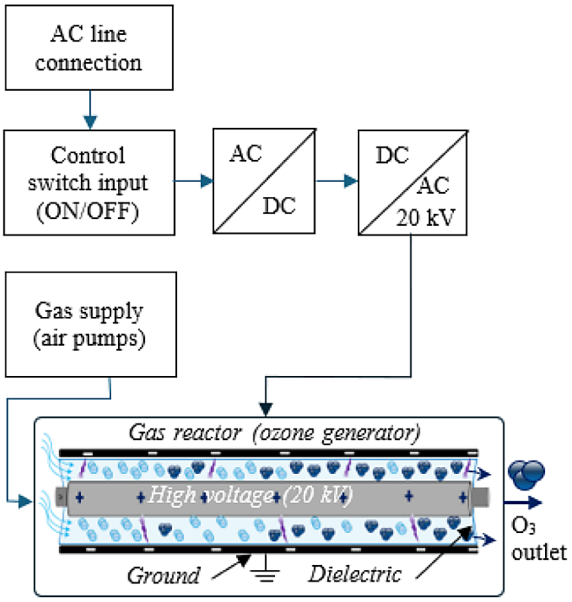

Figure 2. Diagram of the ozone generation process by corona discharge

where: v_1 is the velocity of the fluid flowing through the larger diameter section of the inlet (A_1), and v_2 is the velocity of the fluid flowing through the smaller diameter section (A_{12}) of the Venturi tube.

Bernoulli's principle on the other hand establishes the principle of conservation of energy. The energy contained in a fluid in a closed circuit is the sum of kinetic, potential and pressure energy (Equation 3), which must remain constant at the inlet and outlet of the circuit (Figure 4).

$$p_1 + \rho g y_1 + \frac{1}{2}\rho v_1^2 = \\ = p_2 + \rho g y_2 + \frac{1}{2}\rho v_2^2 \quad (3)$$

where: y_1, v_1 and v_1 represent, respectively, the height, velocity and pressure of the fluid in the larger diameter section of the Venturi tube. Similarly, y_2, v_{21} and v_2 correspond to those parameters in the smaller diameter section. ρ is the density of the fluid which is kept constant, and g is the acceleration due to gravity.

By removing the potential energy (Equation 3) by remaining constant, Equation 4 is obtained.

$$p_1 + \frac{1}{2}\rho v_1^2 = p_2 + \frac{1}{2}\rho v_2^2 \quad (4)$$

Knowing the pressure variation, we can obtain the velocity variation experienced by the fluid (Equation 5).

$$v_1^2 - v_2^2 = \frac{2(p_2 - p_1)}{\rho} \quad (5)$$

As the flow rate (Q) is constant in the circuit, it obeys Equation 6. In gas injection, this formula is used to determine the flow rate of the liquid flowing through the Venturi; the pressure drop between p_1 and p_2 generates a partial vacuum in the throat, which allows the gas to be sucked from a lateral line, even without the need for pumping or additional compressors.

$$Q = A_1 A_2 \sqrt{\frac{2(p_2 - p_1)}{\rho(A_1^2 - A_2^2)}} \quad (6)$$

Level sensor

To measure the water level in the process tanks, the SITRANS Probe LU 2-wire level transmitter sensor (Figure 5) was used, which provides

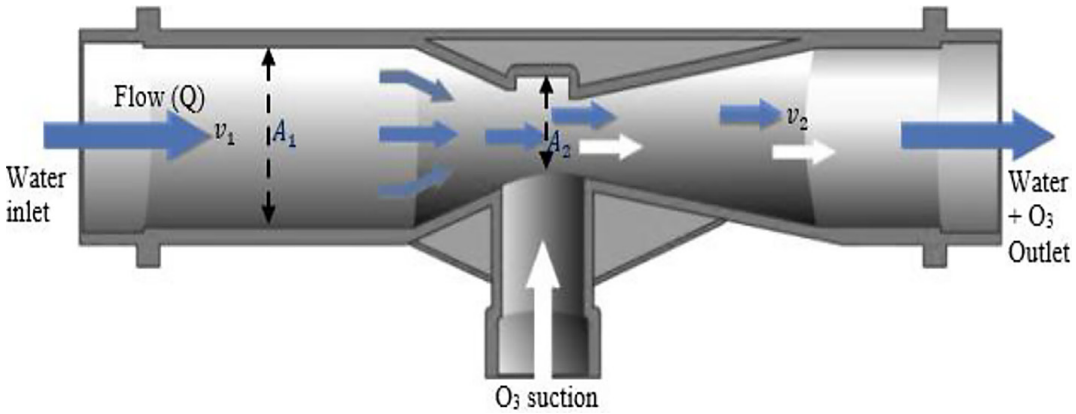

Figure 3. Venturi tube used to inject ozone into water

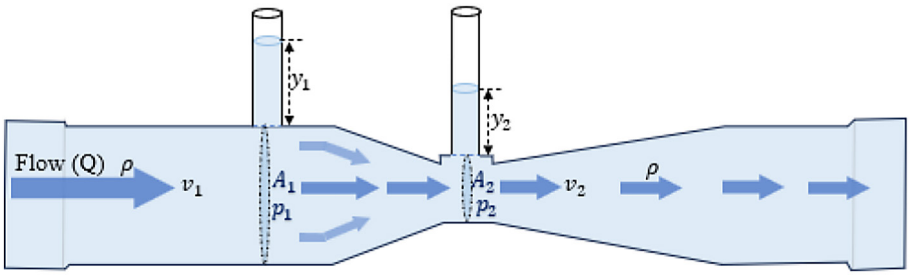

Figure 4. Representation of Bernoulli's principle in a Venturi tube

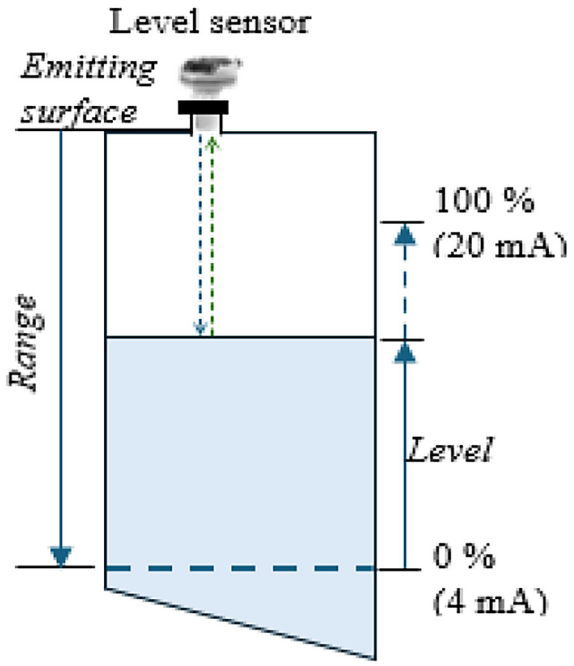

Figure 5. Level sensor current range and equivalence

a 4–20 mA current proportional to the level set according to the depth of the tanks and the set range, this device is designed for continuous ultrasonic level measurement (Gao et al., 2021).

ORP and pH sensors

One of the important elements for the implementation of the controller was the ORP sensor (ASTM, 2022), with an accuracy of 0.01 mV, measuring range of ± 2.000 mV with a degree of protection for immersion in water (IP 68), which was installed directly in the pipe (Figure 6a).

Likewise, the same installation was made of a robust pH sensor installed for online measurements on the pipe (Nair et al., 2024), consisting of a passive bulb that detects the current generated in the presence of H^+ in solutions, with a measurement range of 0 to 14. In this work for the connection to the PLC a pH sensor was used together with the 4 mA to 20 mA signal transmitter, with IP 68 protection, which is submersible in water withstanding temperatures from 0 to 60 °C, the installation was also direct in the pipeline (Figure 6b).

Control sequence

The flow diagram in Figure 7 represents the stages of the ozonation water treatment control process, which begins with the definition of input parameters: water volume (L),

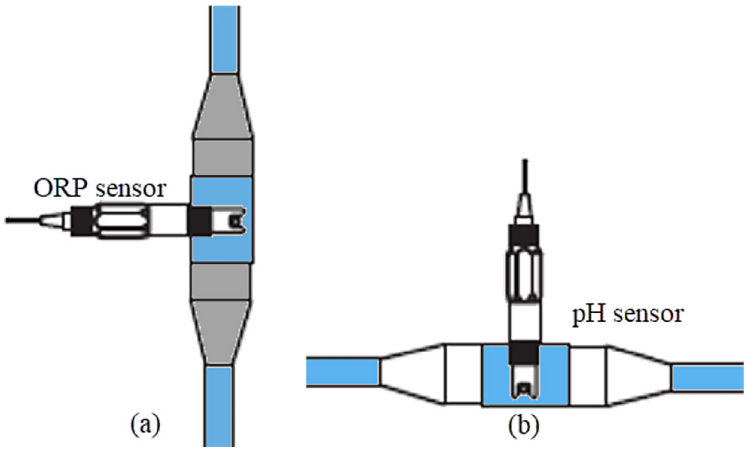

Figure 6. Pipeline installation: (a) ORP sensor and (b) pH sensor

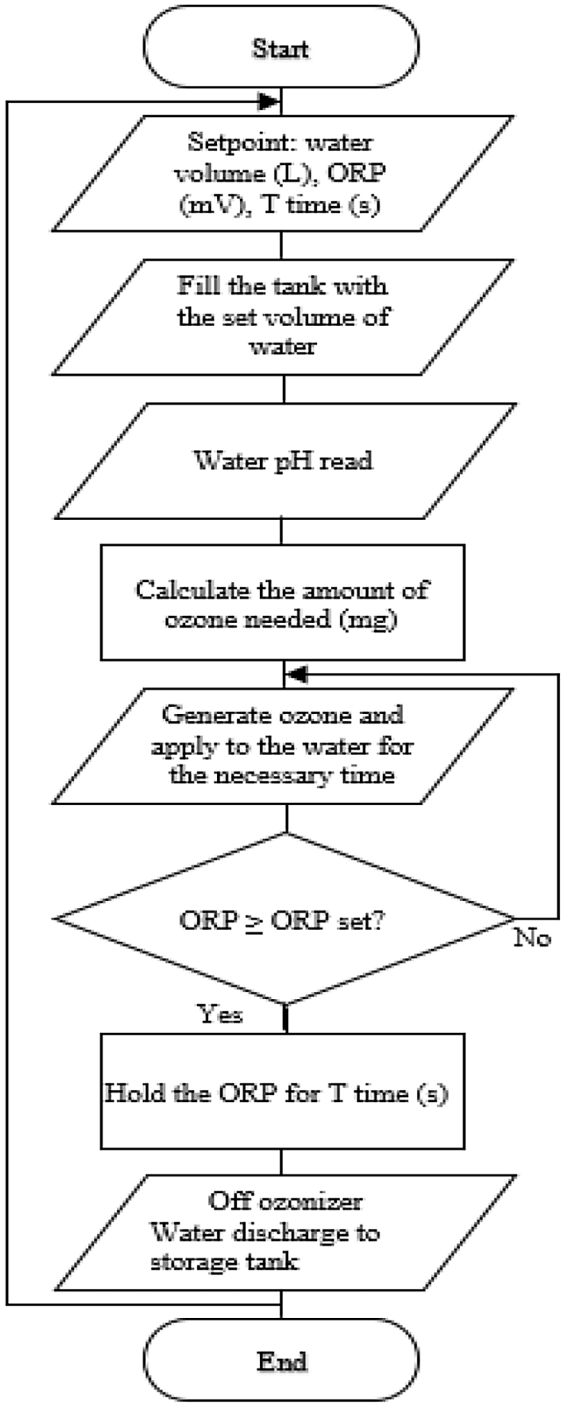

Figure 7. Flow chart of ORP control sequence for bacterial disinfection of water

oxidation-reduction potential target value (ORP in mV) and retention time (T in seconds). The tank is then filled with the set volume and a pH reading of the water is taken to calculate the amount of ozone required. Ozone is then generated and applied to the water for the required time until the desired ORP is reached. Once this value is reached, the system maintains this ORP for the predefined time T. Finally, the ozonizer is turned off and the treated water is discharged to the storage tank, concluding the process.

The block and piping diagram shown in Figure 8 represents the operation of the ozone-induced ORP control system, based on a Siemens S7-1500 PLC as the main controller element. The process starts with the entry of water through a filling valve, regulated by a signal from the controller based on the level sensor located in Tank 1 (Tank1 - Process). Once the desired level is reached, the pH measurement is activated by a sensor, whose reading is used to calculate the required ozone dosage. Ozone is generated by an ozonizer, which in turn receives concentrated oxygen from an O_2 concentrator, and is injected into the water through a Venturi type injector, with a check valve system to prevent fluid backflow. An ORP sensor monitors the redox potential of the water in real time. If the ORP value is below the set value, the PLC continues to activate the ozone generator. When the ORP reaches the set point, the system holds it for a set time. Then, a discharge valve is activated to allow the treated water to flow into Tank 2 (Tank2 - Storage). Finally, the stored water can be released through an outlet valve. The contactor and pump allow the recirculation or dosing of water within the system. The entire process is monitored and controlled by the PLC control panel, which receives and sends signals to all components to ensure efficient and safe treatment of the water by ozonation.

Figure 9 shows a fragment of the program developed in the TIA Portal environment for the Siemens S7-1500 PLC, intended for the automated control of the ozonation process through ORP regulation. The code is written in structured high-level language (Structured Text - ST) and is based on a sequential logic that regulates different stages of the process.

The program starts with a start condition (Start_button) that activates the first stage of the cycle (Step0), as long as the stop button is not active. From there, transition conditions are defined between the different steps of the process (Step0 to Step6), controlled by sensor signals, setpoints, ORP levels, water volume and programmed times. Step1 validates that the volume of water in the tank has reached the setpoint to proceed to the next step. In Step3, it is evaluated if the current ORP value exceeds the setpoint value, activating the ORP holding logic by means of a timer (TP_Timer_0_0_PB) that regulates the ozonation holding time.

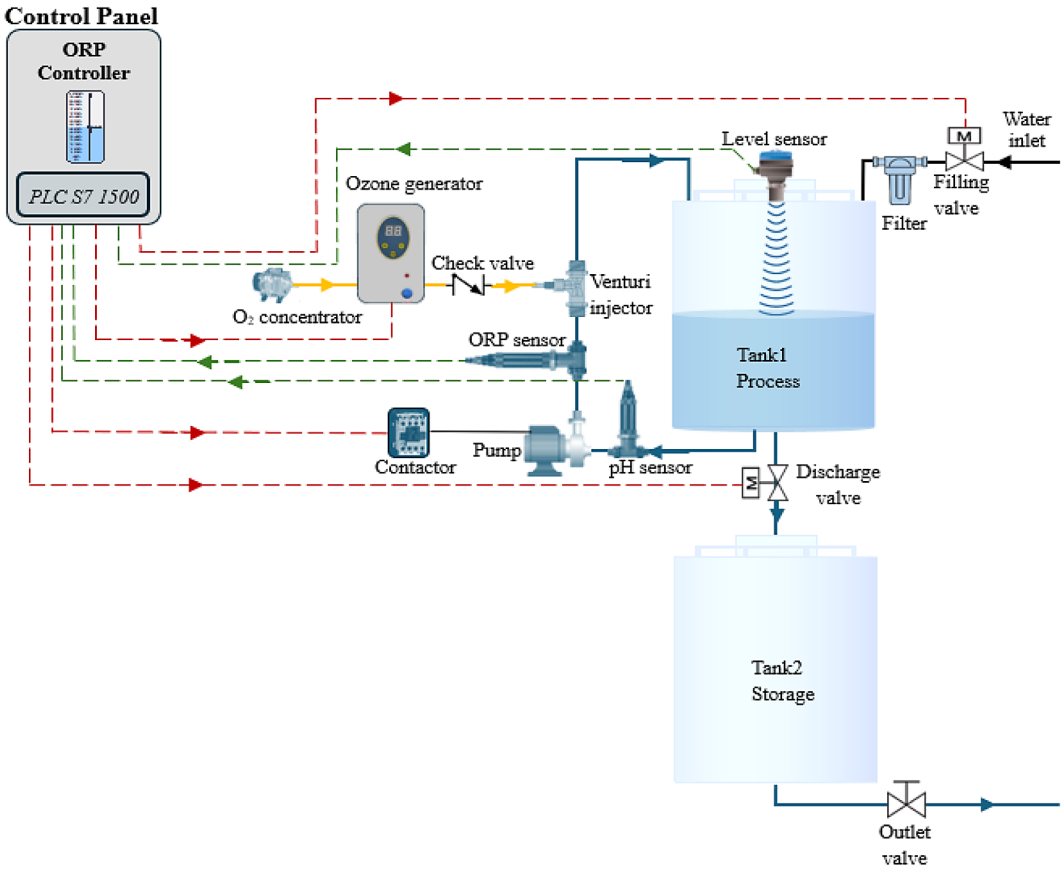

Figure 8. Block and piping diagram of the ozone-induced ORP controller

In addition, the code provides for safety mechanisms such as emergency shutdown and output deactivation (Ozonizer_Relay) if the conditions are not met. The sequence ends with Step6, which indicates the end of the cycle and prepares the system for a new process.

The HMI interface of the ozone-induced ORP controller, implemented on a TP700 Comfort panel using SIMATIC WinCC Runtime Advanced (Yu, 2024), is presented in Figure 10, allowing intuitive monitoring and control of the water treatment system controlled with an S7-1500 PLC. On the left side are the control buttons (Start and Stop/Reset), as well as the PLC status display. Two main panels are included: one for the input of setpoint values such as water volume, ORP, ozonation time and ozone dose, and the other for the real-time display of these measured variables, including pH. The central process diagram graphically represents the system components, such as the ozone generator, Venturi, process and storage tanks (TANK1 and TANK2), valves, sensors and water flow, which facilitates the understanding of the system operation. The interface also features navigation buttons and labels that allow safe and efficient operation, providing the operator with a complete interface for monitoring and adjusting the ozonation process based on ORP control.

RESULTS AND DISCUSSION

The results presented in Table 1 correspond to the design of experiments (DOE) used to evaluate the effects of ORP and ozonation time on the microbial inactivation of *Escherichia coli* O157:H7, which is reproducible in water (Mohseni et al., 2022), and *Total coliform* in 100 ml samples of drinking water with a pH of 6.8, maintained across all experiments. Two factors were analyzed: ORP, ranging from 550 to 901 mV, and ozonation time, ranging from 5 to 210 seconds. The measured responses were the concentrations of *E. coli* and *Total coliform*, expressed in MPN/100 ml. The experimental design included 13 runs, with 5 central point replications (ORP: 725 mV; Ozonation Time: 105 s). The estimated experimental ranges align with those reported by Suslow (2025), who found that an ORP above 650 mV generates an oxidative environment strong

```
REGION STEPS
IF "FirstScan1" OR ("Step6" AND "Cycle_end")OR "Data_Block".Stop_buttom THEN
    // Process initialization
    "Start_On" := FALSE;
    "Step0":= TRUE;
    "Step1" := FALSE;
    "Step2" := FALSE;
    "Step3" := FALSE;
    "Step4" := FALSE;
    "Step5" := FALSE;
    "Step6" := FALSE;
END_IF;

IF "Step0" AND "Data_Block".Start_buttom THEN
    // Step1
    "Start_On" := TRUE;
    "Step1" := TRUE;
    "Step0" := FALSE;
END_IF;

IF "Step1" AND ("Data_Block".Setpoint_water_volume > 0 AND
    "Data_Block".Current_water_volume >= "Data_Block".Setpoint_water_volume) THEN
    // Step2
    "Step2" := TRUE;
    "Step1" := FALSE;
END_IF;

"IEC_Timer_0_DB_1".TON(IN := "Step2",
                       PT := "Data_Block".Ozonization_Time,
                       Q => "TEMPO1");

IF "Step2" AND ("Data_Block".Current_ORP>="Data_Block".Setpoint_ORP) THEN
    // Step3
    "Step3" := TRUE;
    "Step2" := FALSE;
END_IF;

IF "Step3" AND "Data_Block".Current_ORP > "Data_Block".Setpoint_ORP  THEN
    // Step4
    "Data_Block".Warning := TRUE;
    "Ozoniser_Relay" := FALSE;
    "Step4" := TRUE;
    "Step3" := FALSE;
ELSIF "Step3" AND "Data_Block".Current_ORP = "Data_Block".Setpoint_ORP  THEN
    "Data_Block".Warning := FALSE;
    "Ozoniser_Relay" := TRUE;
    "Step4" := TRUE;
    "Step3" := FALSE;
END_IF;

"IEC_Timer_0_DB_2".TON(IN:="Step4",
                       PT:="Data_Block".Set_Ozonization_Time,
                       Q=>"TEMPO2");

IF "Step4" AND "TEMPO2" THEN
    // Step5
    "Step5" := TRUE;
    "Step4" := FALSE;
END_IF;

IF "Step5" AND "Data_Block".Current_water_volume <=0 THEN
    // Step6
    "Step6" := TRUE;
    "Step5" := FALSE;
END_IF;

"IEC_Timer_0_DB_3".TON(IN:="Step6",
                       PT:=T#2S,
                       Q=>"Cycle_end");

END_REGION
```

Figure 9. Program developed for S7-1500 PLC of the ozone induced ORP controller

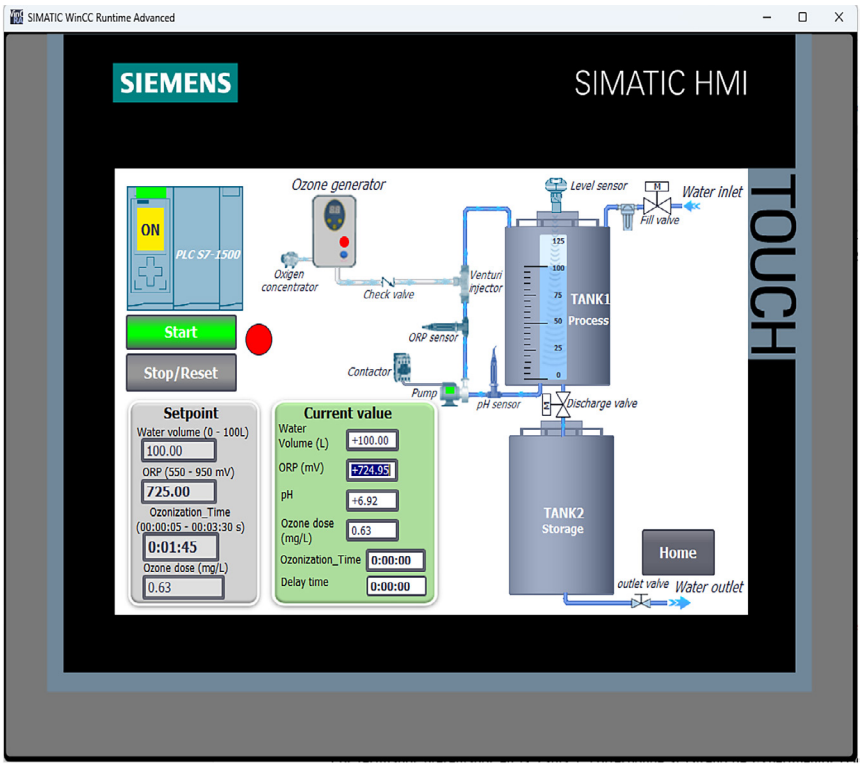

Figure 10. Operation of the HMI interface of the ozone induced ORP controller implemented in TP700 Comfort

enough to cause irreversible damage to the cell membranes of *E. coli* O157:H7 and other pathogenic bacteria in aqueous environments, with a minimum exposure time of 30 seconds.

Coliform Agar, a culture medium formulated in accordance with the composition specified in ISO 9308-1, was used for microbiological analysis. In these medium, coliform bacteria form pink to red colonies, while *Escherichia coli* develops blue to purple colonies, enabling clear differentiation and accurate enumeration during sample analysis. This specialized medium is widely recognized for its effectiveness in detecting and quantifying coliform bacteria in a variety of matrices, including water and food products (Azuga et al., 2025; Banseka and Tume, 2024).

Regarding the response of *Escherichia coli*, the highest contamination levels (370 and 798 MPN/100 ml) were observed under conditions of low ORP (600 and 725 mV) and short ozonation times (5 and 30 seconds). As ORP values and ozone exposure times increased, the *E. coli* concentration decreased significantly, reaching zero in multiple runs, indicating complete inactivation of the microorganism. This trend suggests that an ORP equal to or greater than 725 mV combined with ozonation times of at least 105 seconds is effective for water disinfection. These results are consistent with findings by Lou et al. (2024) and Cabral et al. (2023), who also reported that the inactivation kinetics of *E. coli* by ozone are more strongly influenced by contact time than by absolute redox potential. Similarly, Haghighi et al. (2020) found that in water systems intended for medical use, ozone exposure time was the most critical parameter for microbial reduction, achieving over 99% efficiency with exposure times exceeding 120 seconds. Moreover, Xue et al. (2023) reported that in ozone-based disinfection systems, higher ORP values and longer ozonation durations significantly reduced *E. coli* concentrations, achieving complete inactivation at ORP levels above 725 mV and exposure times of at least 100 seconds.

Similarly, the concentration of *Total coliform* exhibited a decreasing trend as ORP levels and ozonation times increased. The highest recorded value (1100 MPN/100 ml) corresponded to an ORP of 725 mV and an ozonation time of 5 seconds, suggesting that despite a relatively high ORP, insufficient contact time limits the effectiveness of the disinfection process. In contrast, runs with higher ORP values (850–901 mV) and ozonation times exceeding 105 seconds resulted in complete inactivation of *Total coliform*. These findings are consistent with those of Epelle et al. (2022), who demonstrated that ozone's disinfection efficiency is highly dependent on the oxidant's retention time in the aqueous medium rather than its initial concentration. Furthermore, studies involving more complex matrices, such as wastewater, have reported that while ORP can serve as a useful indirect indicator of disinfection, its direct effect is limited unless accompanied by adequate exposure time (Contreras-Soto et al., 2025).

The analysis of variance (ANOVA) applied to the model evaluating the presence of *E. coli* (Table 2) shows that ozonation time has a statistically significant impact ($p = 0.0061$) on bacterial reduction, with a nonlinear effect evidenced by the significance of the quadratic term (B^2, $p = 0.0206$). Although the overall model is significant ($p = 0.0241$), the lack of fit is highly significant ($p < 0.0001$), suggesting that additional factors may be needed to improve predictive accuracy. The ORP variable (A) and the interaction term between A and B were not statistically significant, while the low pure error variance (2.80) indicates high precision in the measurements. Therefore, the model for *E. coli* removal requires further adjustment of factors A and B to enhance optimization. As noted by Joshi and Kumari (2023), it is necessary to

Table 1. Responses of the 13 *E. coli* and *Total coliform* treatments with ORP and Ozonization_Time factors

Std	Run	Factor 1 A: ORP mV	Factor 2 B: Ozonization_Time *s*	Response 1 *E. coli* MPN/100 ml	Response 2 *Total coliform* NMP/100 ml
0	0	-	0	2800	4600
1	1	600	30	370	420
2	2	850	30	12	18
3	3	600	180	1	2
4	4	850	180	0	0
5	5	550	105	170	189
6	6	901	105	0	0
7	7	725	5	798	1100
8	8	725	210	0	0
9	9	725	105	2	2
10	10	725	105	1	1
11	11	725	105	1	1
12	12	725	105	0	1
13	13	725	105	0	0

Table 2. ANOVA for the quadratic model of *E. coli*

Source	Sum of squares	df	Mean square	F-value	p-value	
Model	5.245E+05	5	1.049E+05	5.36	0.0241	significant
A-ORP	44884.18	1	44884.18	2.29	0.1737	
B-Ozonization_ Time	2.930E+05	1	2.930E+05	14.97	0.0061	
AB	31862.25	1	31862.25	1.63	0.2427	
A^2	2.51	1	2.51	0.0001	0.9913	
B^2	1.733E+05	1	1.733E+05	8.85	0.0206	
Residual	1.370E+05	7	19568.81			
Lack of fit	1.370E+05	3	45659.62	65228.03	< 0.0001	significant
Pure error	2.80	4	0.7000			

consider the contribution of ozone-derived compounds, such as hydroxyl radicals generated upon ozone decomposition in water, which represent the most powerful oxidants available for water treatment (Cao et al., 2022).

Equation 7 represents the concentration of *E. coli,* where it shows that the ozonation time (*B*) is the most influential factor in its reduction, with a significant negative coefficient (-15.6878), followed by ORP (*A*), which also contributes to reduce the presence of the bacteria (-1.65721).

$$E.Coli = 1784.97 - 1.65721 \cdot \mathrm{A} - \\ -15.6878 \cdot \mathrm{B} + 0.00952 \cdot \mathrm{AB} + \\ + 0.0000387657 \cdot A^2 + 0.0294688 \cdot B^2 \quad (7)$$

Figure 11 presents the three-dimensional response surface of the interaction between ORP and Ozonization_Time on the concentration of *E. coli* in drinking water. The color scale, which varies from blue (0 MPN/100 ml) to red (798 MPN/100 ml), allows the identification of areas of higher and lower bacterial presence. The design points, differentiated by color, show the experimental distribution of the data, indicating values above and below the adjusted surface. The results reflect that a longer ozonation time contributes significantly to the elimination of *E. coli*, while ORP has a less pronounced influence in the range analyzed.

The analysis of variance for *Total coliform*, presented in Table 3, indicates that ozonation time has a statistically significant effect (p = 0.0102) on bacterial reduction, with a nonlinear trend supported by the significance of the quadratic term (B^2, p = 0.0282). Although the overall model is statistically significant (p = 0.0421), the lack of fit is highly significant (p < 0.0001), suggesting that the model does not fully capture the variability in the data and may require refinement. The ORP variable (A) and the interaction between A and B were not statistically significant, while the low pure error variance (2.00) reflects high precision in the experimental measurements. Therefore, the model for *Total coliform* removal requires further adjustment of factors A and B to achieve optimal performance. These findings are consistent with the study by Panigrahi et al. (2021), which modeled the inactivation kinetics of *Total coliform* during ozone treatment of liquids and identified treatment time as the most influential factor, necessitating extended exposure. Furthermore, according to U.S. FDA regulations, the residual ozone concentration in drinking water should not exceed 0.4 mg/L (Lou et al., 2024).

Equation 8 represents the concentration of *Total coliform,* where it shows that the ozonation time (*B*) is the most influential factor in its reduction, with a significant negative coefficient (-19.5574), followed by ORP (*A*), which also contributes to reduce the presence of the bacteria (-0.556516).

$$Total\ Coliform = 1661.18 - \\ - 0.556516 \cdot \mathrm{A} - 19.5574 \cdot \mathrm{B} + 0.0106667 \cdot \mathrm{AB} - \\ - 0.000852665 \cdot A^2 + 0.039998 \cdot B^2 \quad (8)$$

Figure 12 presents the three-dimensional response surface of the interaction between ORP and Ozonization_Time on the concentration of *Total coliform* in drinking water. The color scale, which varies from blue (0 MPN/100 ml) to red (1100 MPN/100 ml), allows the identification of areas of higher and lower bacterial presence. The design points, differentiated by colors, show the experimental distribution of the data, indicating values above and below the adjusted surface. The

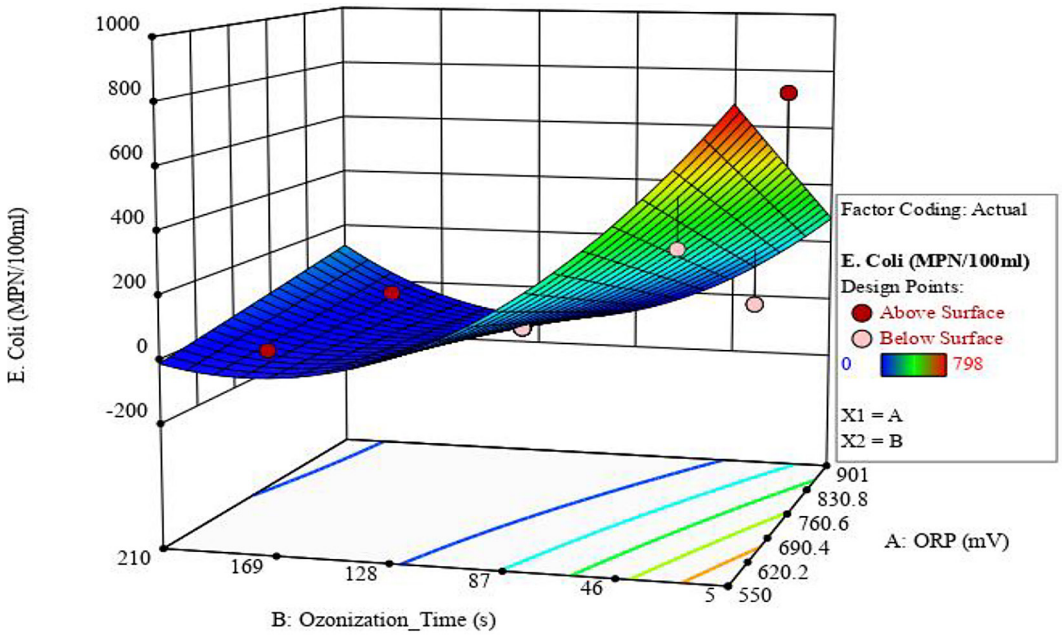

Figure 11. Three-dimensional response surface of the interaction between ORP and Ozonization_Time on *E. coli* concentration

Table 3. ANOVA for the quadratic model of *Total coliform*

Source	Sum of squares	df	Mean square	F-value	p-value	
Model	8.974E+05	5	1.795E+05	4.28	0.0421	significant
A-ORP	56187.17	1	56187.17	1.34	0.2852	
B-Ozonization_ Time	5.092E+05	1	5.092E+05	12.13	0.0102	
AB	40000.00	1	40000.00	0.9530	0.3615	
A^2	1213.44	1	1213.44	0.0289	0.8698	
B^2	3.192E+05	1	3.192E+05	7.61	0.0282	
Residual	2.938E+05	7	41972.61			
Lack of fit	2.938E+05	3	97935.41	1.959E+05	< 0.0001	significant
Pure error	2.00	4	0.5000			
Cor total	1.191E+06	12				

results show that a longer ozonation time contributes significantly to the elimination of *Total coliform*, while the ORP has an almost constant influence in the range analyzed.

On the other hand, Table 4 presents the optimal parameters, calculated using Design-Expert 13 software, for the elimination of *E. coli* and *Total coliform* through ozonation. The identified optimal conditions were an ORP of 719 mV and an ozonation time of 110 seconds. Under these conditions, the concentration of both bacterial groups was reduced to 0.000 MPN/100 ml, indicating complete inactivation. The associated standard errors (62.507 for *E. coli* and 91.544 for *Total coliform*) reflect variability in the experimental data; however, the high desirability value (1.000) suggests that these conditions are ideal within the optimized model, contributing to energy consumption savings during the process. These findings align with results from various studies that have applied response surface methodology for process optimization. Niu et al. (2021) focused on optimizing ozone dosing for drinking water treatment, highlighting the importance of determining the optimal dose to improve water quality while minimizing energy consumption. Similarly, Hogard et al. (2023) optimized bacterial and viral disinfection in water reuse systems by enhancing ORP levels within the range of 650–750 mV and applying ozonation times between 120 and

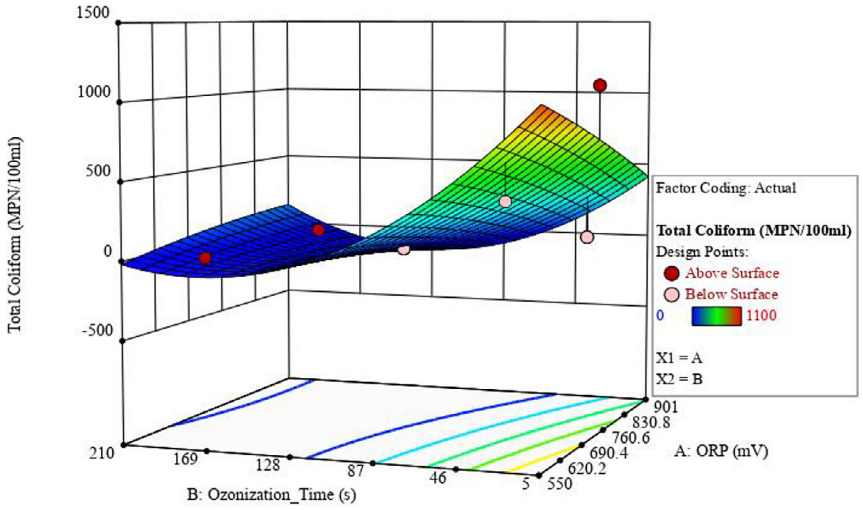

Figure 12. Three-dimensional response surface of the interaction between ORP and Ozonization_Time on *Total coliform* concentration

Table 4. Optimization solution for *E. coli* and *Total coliform* elimination

Number	ORP mV	Ozonization_ Time s	*E. coli* MPN/100 ml	StdErr (*E. coli*)	*Total coliform* MPN/100 ml	StdErr (*Total coliform*)	Desirability
1	719	110	0.000	62.507	0.000	91.544	1.000

180 seconds. Additionally, Haghighi et al. (2020) and Bu et al. (2021) successfully applied RSM to optimize variable interactions, identifying optimal conditions for maximum bacterial reduction while ensuring compliance with health standards.

CONCLUSIONS

As a key component of this study on the optimization of bacterial disinfection in drinking water through ozone-induced ORP control and response surface methodology, an automated ORP control system was successfully implemented using a Siemens S7-1500 PLC with an HMI TP700 Comfort display. This system ensured precise automation and regulation of the critical process parameters across the 13 experimental treatments, providing safe, consistent, and repeatable operational control. The integration of automation technology represents a significant advancement in water treatment management, enabling real-time monitoring and dynamic parameter adjustments to maximize disinfection efficiency.

The results demonstrated that ozonation time is the most influential factor in bacterial reduction, with significant inactivation of *E. coli* and *Total coliform* observed at exposure times exceeding 105 seconds and ORP values above 725 mV. These findings underscore the importance of establishing accurate operational parameters to guarantee the effectiveness of the disinfection process. Although the interaction between ORP and ozonation time had a lesser statistical impact, the significance of the quadratic term revealed a nonlinear relationship in the bacterial inactivation process, indicating that further increases in ORP or ozonation time may produce varying system responses.

Process optimization identified the ideal conditions for complete bacterial removal as an ORP of 719 mV and an ozonation time of 110 seconds, under which a final concentration of 0 MPN/100 ml was achieved in the treated drinking water. These findings are essential for guiding the establishment of microbiological quality standards in water treatment, providing a robust scientific foundation for enhancing current disinfection systems and promoting safer, more efficient, and sustainable approaches to drinking water purification.

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