https://doi.org/10.46813/2025-155-098

STUDY OF BIOCIDAL PROPERTIES OF OZONE, PLASMA-CHEMICAL AND COMBINED TREATMENTS ON ARTIFICIALLY CONTAMINATED MODEL WATER SOLUTIONS

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Study was carried out to evaluate the biocidal effects of plasma-chemical methods for use by ozone, electric discharge with water electrode (plasma-chemical treatment) or their combination of artificially contaminated water. The preliminary contamination was achieved by inoculating water with suspensions of test cultures, including *Escherichia coli* #12 and *Aspergillus flavus*. The results showed that separate exposure to ozone or discharge with water electrode resulted in insignificant fungistatic effect on *Aspergillus flavus* and bacteriostatic effect on *Escherichia coli* #12 after exposure times of 2, 2.5, and 3 h, achieving inhibition rates of about 64%. Joint combined ozone and discharge with water electrode treatment during 2.5…3 h of water contaminated with a mixture of *Escherichia coli* #12 and *Aspergillus flavus* significantly inhibited microorganisms, causing destruction of the cell membrane and genetic material, resulting in the death of 95% of the cells. Such kind of treatment effectively improved the microbiological quality of drinking water.

PACS: 52.80. Hc, 52.90. +z,52.75.−d, 52.77.-g

INTRODUCTION

Purification of drinking water from biological contaminants is one of the most critical safety issues worldwide, as it serves as a primary barrier to pathogens and is crucial for public health protection. Regardless of whether drinking water originates from groundwater or surface sources, it typically undergoes purification before reaching consumers. Even in the cleanest natural water bodies, contaminants such as human and animal waste, and organic matter, can degrade water quality. Modern water treatment methods can reduce most contaminants in source water to permissible levels before distribution. These methods depend on the quality of the initial water and the contaminants it contains [1].

Biocides are antimicrobial substances widely used in households, industry, and healthcare for disinfection. Biocides, like the chlorinated hand wash introduced by 19th-century physician Ignaz Semmelweis, have been integral part in infection control alongside antibiotics.

However, extensive biocide use across different concentrations has significantly increased microbial resistance. Studies have shown that bacteria like *Salmonella typhi* exhibit resistance to chlorine-based biocides, indicating that biocide misuse may cause selective pressure in bacteria, leading them to develop resistance mechanisms.

Thus, finding alternative disinfection methods and agents is an increasingly important area of research. The biocidal potential of ozone (O_3) is gaining particular attention. In vitro microbiological and cellular assays have confirmed the biocidal effects of ozonated water. After brief incubation with ozonated water, bacterial strains and yeast cells lost their proliferative properties.

Dissolved O_3 in water is considered a potent antimicrobial agent due to its strong oxidizing ability, which is effective against both Gram-positive and Gram-negative bacteria, viruses, fungi, and protozoa. Ozone causes bacterial cell lysis by oxidizing membrane phospholipids and lipoproteins, as observed in the Gram-positive membrane of *Listeria monocytogenes* [2].

For deactivating bacteria in water, higher doses of ozone (0.5…3.0 mg/l) are required to achieve a 90…99% reduction in bacterial load, depending on water purity levels. This provides deep inactivation of pathogenic microorganisms, including *Clostridium perfringens*, *Streptococcus faecalis*, *Mycobacterium tuberculosis*, and sanitary indicators like *Escherichia coli*. Bacteria vary in their resistance to ozone; for instance, *Bacillus subtilis*, *Mycobacterium lacticum*, *E. coli*, and *Pseudomonas fluorescens* exhibit relatively high resistance. This resistance is also observed in filamentous fungi like *Aspergillus brasiliensis* and opportunistic yeast *Candida albicans* [3–5].

Water disinfection methods used in water treatment facilities are often ineffective against certain hazardous pathogens. Globally, ozonation is one of the most effective methods for removing organic biological contaminants from water, but its high toxicity and the short-lived nature of its bactericidal effects due to rapid ozone decomposition are drawbacks [6].

Pilot studies have been conducted to determine the individual biocidal effects of ozone and plasma-chemical discharge on viral, bacterial, and fungal infections. The results demonstrated the effectiveness of both methods against viruses and bacteria [7].

Thus, exploring low-toxicity methods combined with ozonation, which would have a synergistic effect on complex pathogenic microorganisms, is highly relevant.

1. EXPERIMENTAL STUDIES

To study the biocidal effectiveness of ozone, plasma-chemical (PC) treatment, and their combined action, pilot experiments were conducted using water treated with artificially introduced test cultures of museum strains of *Escherichia coli* #12 and *Aspergillus flavus* (A.X.5).

The experiments were carried out on two specially designed and constructed experimental setups for ozone and plasma-chemical treatment of solutions. Ozone in laboratory air was generated using a barrier-free ozone generator. The resulting air-ozone mixture was then bubbled through the solution. In the plasma-chemical treatment setup, the gas discharge was ignited directly above the solution surface, meaning the solution acted as the anode.

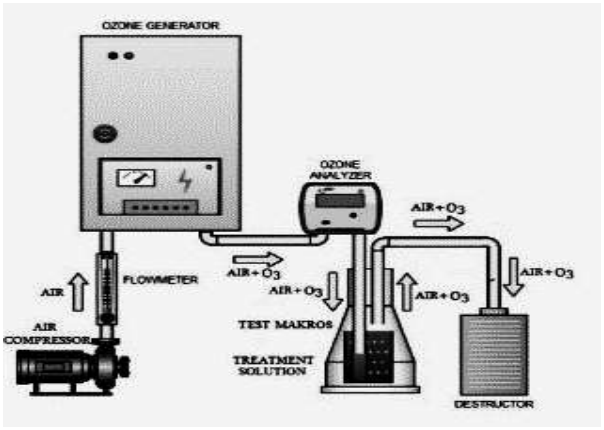

Fig. 1. Ozone treatment system for the studied solution

First experimental setup (Fig. 1) consisted of the following functional blocks: air compressor: Secoh Sangyo (Japan) with a capacity of 50 l/min and a maximum pressure of up to 12.7 kPa, gas flow meter: type RM-4 GU3, laboratory ozone generator: "Streamozone", ozone concentration meter: Teledyne instruments (USA) model 454 h with a measurement range of 0.1…100 g/m³, laboratory macras: for placing the solution samples under study, ozone destructor. he volume of the solution in the bubbling container was 2 l. The concentration of ozone in the ozone-air mixture was 5 g/m³, with a mixture flow rate of 8 l/min

Second experimental setup (Fig. 2) consisted of a specially developed plasma-chemical reactor for generating discharge with one liquid electrode, as shown in Fig. 3. The setup also included: pulse high-voltage power supply: maximum power of 150 W·h, water pump: 1000-ZB with a capacity of 60 l/h, air compressor: SunSun ACO-003 with a capacity of 38 l/min to create an airflow through the discharge gap, laboratory macras: for placing the solution samples under study, ozone destructor.

The high-voltage pulse power supply generated high-voltage pulses lasting about 1300 ns with a pulse repetition frequency of 1…15 kHz and an amplitude of up to 12 kV. The pulse rise time was 500 ns. The volume of the solution in the system was 4 l.

The volume of the solution in the aerated container was 2 l. The concentration of ozone in the air-ozone mixture was 5 g/m³ with a flow rate of 8 l/min.

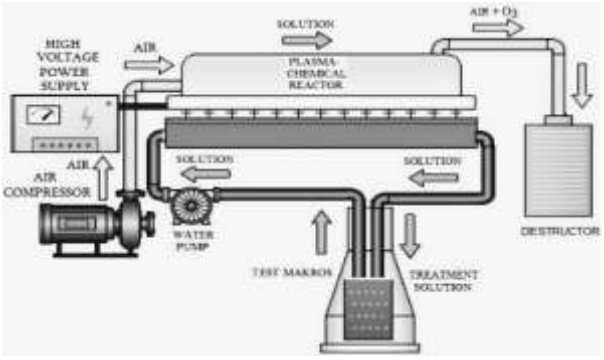

Fig. 2. System of plasma-chemical treatment by discharge with water electrode

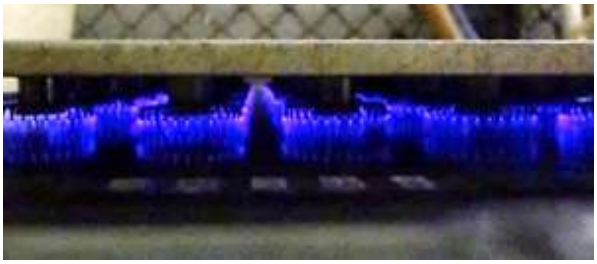

Fig. 3. Photo of plasma-chemical treatment by discharge with water electrode

To ensure a fair comparison of the experimental results regarding the biocidal effectiveness of these treatment methods, a parameter of equal power input per unit volume of the solution at a level of 20 W/l was selected and maintained consistently throughout the experiment.

The biocidal action was determined using separate and combined methods of ozone bubbling through the aqueous solution and high-voltage discharge on the liquid surface. The solution volumes were 2 and 4 l, respectively, prepared from combined working suspensions of each test culture. The experiments were conducted at room temperature (18.5±0.5) °C with exposure durations of 1, 1.5, 2, 2.5, and 3 h.

Results were recorded using parallel bacterial plating of inoculum samples subjected to ozone, plasma-chemical discharge, and their combined action for the specified exposure times. A control sample of bacterial inoculum, taken the day before the experiment and kept unchanged, was used for comparison.

Escherichia coli #12 was plated on nutrient agar and incubated in a thermostat for 24 h. Colony counts were performed after 24 h based on the number of *E. coli* colonies on the Petri dishes grown on nutrient media. If no growth was observed, the Petri dishes were kept in the thermostat for up to 48 h.

Bactericidal activity was confirmed by the absence of test strain bacterial growth on nutrient media, provided there was intensive growth in the corresponding controls [8].

Preparation of *Aspergillus flavus* test culture samples for testing was carried out according to generally accepted and developed laboratory mycological analysis methods [9–12].

Since the experimental water samples were artificially contaminated with a combined suspension of test cultures *Escherichia coli* #12 and *Aspergillus flavus* (A.X.5), when preparing the negative control, in addition to adding nystatin (100 thousand CFU per 100 cm³ of nutrient medium) to the nutrient medium (Chapeck agar), antibiotics from different groups streptomycin and ampicillin (40 thousand CFU per 100 cm³ of nutrient medium)-were additionally added to suppress the microflora. To count the number of spores surviving the treatment time, 3.0 cm³ of the experimental solution was plated onto labeled Petri dishes with Chapeck agar nutrient medium and incubated at temperatures of 25…27 °C. Colony counts were conducted on the 3rd, 5th, and 7th days. After cultivation, macroscopic examination of the cultures was performed within the specified time frames, and comparisons were made with colonies of museum strains. Effective results were considered those parameters where spore mortality of test cultures was 95…98%, provided there was growth in the control [13].

2. RESULTS OF THE STUDY

The study results showed that the bacteriostatic properties of ozone were observed against the test strain *E. coli #12* only after exposure durations of 2, 2.5, and 3 h (65 CFU/ml), while plasma-chemical treatment showed bacteriostatic effects only after 3 h (138 CFU/ml) compared to the control, which confirmed continuous microorganism growth on nutrient media (Table 1). The bacteriostatic action of ozone and plasma-chemical treatment, as well as their combined application, demonstrated a prolonged effect for up to 4 days. Starting from the 5th day, *E. coli #12* growth began to recover. The control used untreated water contaminated with *E. coli #12*.

The experimental museum strain *E. coli* #12 at a microbial body concentration of $1.5 \cdot 10^3$ showed resistance when treated separately with ozone and plasma-chemical treatment at exposures of 1 and 1.5 h, similar to the control, confirmed by the growth of more than 300 CFU/ml on nutrient media.

Table 1

Results of microbiological studies of drinking water, artificially contaminated with *E. coli #12*, treated with ozone, discharge with water electrode and their combined application

№	Exposure, h	Type of Treatment								
		Ozone			Plasma-Chemical treatment			Combined ozone and PCT		
		Terms for *E. coli* Colony Growth (days)								
		1	3	5	1	3	5	1	3	5
1	1	356	368	371	409	409	421	416	416	429
2	1.5	321	339	345	395	395	403	267	267	405
3	2	262	262	267	437	437	442	285	285	394
4	2.5	234	234	239	419	419	423	48	48	337
5	3	273	273	310	258	258	264	42	42	312
Control	–	386	394	403	442	442	458	429	433	447

Table 2

Results of microbiological studies of drinking water, artificially contaminated with *A. flavus*, treated with ozone, discharge with water electrode and their combined application

№ Sample and Exposure Time	Ozone			Plasma-Chemical treatment			Combined ozone and PCT		
	Terms for *A. flavus* Colony Growth (days)								
	3	5	7	3	5	7	3	5	7
Sample № 1 – 1 h	+	+	+	+	+	+	+	+	+
Sample №2 – 1.5 h	+	+	+	+	+	+	+	+	+
Sample № 3 – 2 h	90	93	94	150	170	220	20	22	24
Sample №4 – 2.5 h	78	79	80	130	160	190	11	13	15
Sample № 5 – 3 h	70	72	72	120	150	170	6	7	7
Control Positive	+	+	+	+	+	+	+	+	+
Control Negative	–	–	–	–	–	–	–	–	–
Note: 1 – "–" indicates no growth; 2 – "+" indicates continuous growth.									

Significantly greater reduction in the number of *E. coli* was observed with the combined application of ozone and PC starting from an exposure of 1.5 h, with pronounced inhibition at exposures of 2.5 h (48 CFU/ml) and 3 h (42 CFU/ml) on nutrient media, compared to continuous *A. flavus* growth in the positive control.

When studying the fungicidal (fungistatic) properties of ozone and plasma-chemical treatment for

treating water contaminated with a standardized spore count of the test culture *Aspergillus flavus* at temperatures of (18.0±0.5) °C and exposure times of 1, 1.5, 2, 2.5, and 3 h, the results presented in Table 2 were obtained. Untreated water with *A. flavus* culture was used as a positive control, and untreated water with *A. flavus* culture plus added nystatin, streptomycin, and ampicillin was used as a negative control.

When determining the fungicidal (fungistatic) effect of the plasma-chemical treatment, it was found that this treatment did not affect the inhibition of test culture growth in water-growth of the *A. flavus* test culture was observed, with more than 100 colonies in all experimental exposure times.

Fungicidal properties were observed only with the combined treatment of ozone and plasma-chemical treatment at exposure times of 2, 2.5, and 3 h, which can be explained by a synergistic effect. The number of colonies that grew-24, 15, and 7, respectively-compared to the full growth of *A. flavus* in the positive control and its absence in the negative control, demonstrates the effective action of the combined ozone and plasma-chemical treatment on aqueous solutions contaminated with fungal infection.

CONCLUSIONS

The study investigated the biocidal effects of ozone, plasma-chemical treatment, and their combined action on artificially contaminated water using a combined inoculum of test cultures of museum strains *Escherichia coli* #12 and *Aspergillus flavus*.

The individual application of ozone and plasma-chemical treatment exhibited minimal fungistatic properties against *Aspergillus flavus* and bacteriostatic properties against *Escherichia coli* #12 after exposure times of 2, 2.5, and 3 h, achieving an inhibition level of 64%.

Combined treatment with ozone and plasma-chemical treatment of water artificially contaminated with *Escherichia coli* #12 and pathogenic *Aspergillus flavus* for exposure times of 2.5 to 3 h inhibited microorganisms by causing membrane and genetic material destruction, leading to 95% cell mortality (a tenfold reduction) and improving the microbiological quality of drinking water.

The study is supported by the National Research Foundation of Ukraine under the Program “Science to Strengthen Defense Capabilities of Ukraine” (Project #2023.04/0092).

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Article received 29.11.2024
Article accepted 21.01.2025

ДОСЛІДЖЕННЯ БІОЦИДНОЇ ВЛАСТИВОСТІ ОЗОНОВОЇ, ПЛАЗМОХІМІЧНОЇ ТА СУМІСНОЇ ДІЇ ШТУЧНО КОНТАМІНОВАНИХ МІКО- ТА МІКРОФЛОРОЮ МОДЕЛЬНИХ ВОДНИХ РОЗЧИНІВ

І.М. Оніщенко, Г.В. Таран, О.О. Замурієв, П.О. Опалєв, М.О. Ярошенко, О.В. Кольчик

Проведено дослідження оцінки біоцидних ефектів плазмохімічних методів при використанні озону, електричного розряду з водяним електродом (плазмохімічна обробка) або їх комбінації на штучно забрудненій воді. Попереднє забруднення досягали шляхом інокуляції води суспензіями тест-культур штамів кишкової палички *Escherichia coli* # 12 та грибів *Aspergillus flavus*. Показано, що застосування окремо озону та розряду з водяним електродом проявило незначні фунгістатичні дії щодо тест-культури *Aspergillus flavus* та бактеріостатичні властивості відносно *Escherichia coli* # 12 за експозиції 2; 2,5 та 3 год на рівні 64%. Встановлено, що сумісна обробка озоном та розрядом з водяним електродом води, яка штучно забруднена штамами бактерій *Escherichia coli* # 12 та патогенних грибів *Aspergillus flavus* з експозицією 2,5…3 год інгібує мікроорганізми, викликаючи руйнування мембран та генетичного матеріалу, що призводить до 95% загибелі клітин та дозволяє підвищити якість питної води за мікробіологічними показниками.