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KINETICS OF ELECTROLYTIC OZONE GENERATION AND DECOMPOSITION IN FRESH WATER: INFLUENCE OF BACTERIAL CONTAMINATION AND WATER SOURCE

Introduction. *Microbial contamination of water, especially from domestic wastewater, poses significant public health risks. Ozone, a potent oxidant, offers an alternative to conventional disinfectants due to its strong antimicrobial activity. Electrolytic ozonation has gained interest as a safer, more practical approach for generating aqueous ozone without handling gaseous forms.*

The aim of the study. *To evaluate the efficiency of ozone generation via electrolysis in different water types and to investigate ozone decay kinetics in clean and bacteria-contaminated water.*

Research Methods. *Ozonated water was generated electrolytically in tap and spring water. Ozone concentration was measured in real time over 15 minutes. Ozone stability was tested in clean water and in the presence of Staphylococcus aureus ATCC 6538. Ozone levels were monitored over 60 minutes, and all experiments were conducted in triplicate. Data were analyzed using the Student's t-test.*

Results and Discussion. *Ozone generation was significantly higher in tap water (2.98 ± 0.59 mg/L) compared to spring water (2.00 ± 0.42 mg/L), likely due to higher mineral content enhancing electrolysis efficiency. In decomposition experiments, ozone degraded faster in the presence of S. aureus, confirming that microbial and organic presence accelerates ozone consumption.*

Conclusions. *Electrolytic ozonation is an effective method for producing aqueous ozone, with higher efficiency in mineralized water. However, the presence of bacteria significantly accelerates ozone decomposition, underscoring the importance of immediate application after generation in disinfection protocols.*

KEY WORDS: **aqueous ozone; bacterial contamination; electrolytic ozonation; portable ozonator; ozone decomposition.**

INTRODUCTION. Microbial contamination of water sources due to human activity, particularly faecal pollution, remains a serious public health concern, especially in densely populated areas. Water bodies are often affected by microorganisms of both human and animal origin, with domestic wastewater being a significant contributor. Such wastewater contains a variety of microorganisms potentially hazardous to human health [1]. Water treatment processes must ensure the inactivation of these pathogens, and chlorination remains the most commonly used method [2]. Ozone, a powerful oxidizing agent, also possesses strong bactericidal properties and is considered a viable alternative to conventional disinfectants [3]. Due to its oxidative capacity, ozone inactivates microorganisms by denaturing proteins, oxidizing fatty acids, and damaging nucleic acids [4]. Its effectiveness has been demonstrated against planktonic bacterial cells, bacterial biofilms, and even bacterial spores [5]–[7]. Traditionally, ozone is introduced into water via bubbling, using devices that generate gaseous ozone from air or oxygen [1]. This method, however, involves handling gaseous ozone, which poses health risks such as respiratory and ocular irritation [8]. An alternative approach is electrolytic ozonation, where ozone is generated directly in water without the gaseous phase [9]. This technique has gained popularity due to the availability of affordable, portable household ozone generators. Ozone generated through bubbling is relatively unstable in aqueous solution, with a half-life of approximately 30 minutes [10]. As such, freshly ozonated water is recommended for each use [11]. However, our previous studies have shown that electrolytic ozonation results in a more stable solution, with measurable ozone levels persisting for several days [12].

The aim of the present study was to evaluate the efficiency of ozone generation via electrolysis and to examine the kinetics of ozone decay in clean water and in the presence of bacteria, using the reference strain Staphylococcus aureus ATCC 6538.

RESEARCH METHODS. The *Staphylococcus aureus* ATCC 6538 strain, commonly

used for disinfectant efficacy testing [13], was obtained from the culture collection of the Laboratory of Microbiological and Parasitological Research at the I. Horbachevsky Ternopil National Medical University. A bacterial suspension was prepared from a 24-hour culture grown in meat-peptone broth. The culture was centrifuged at 3000 rpm for 5 minutes using an 80-2 Benchtop Universal Laboratory Centrifuge (Jiangsu Jinyi Instrument Technology Co., Changzhou, China). The pellet was resuspended in sterile saline, and the centrifugation step was repeated once to remove residual culture medium. The final pellet was resuspended in sterile saline. Suspension turbidity was standardized to 0.5 McFarland units (approximately 8 log CFU/mL) using a DEN-1 densitometer (BioSan SIA, Riga, Latvia).

Aqueous ozone solutions were prepared by direct electrolysis of two types of freshwater: tap water (Ternopil city, microdistrict "Center") and spring water (Hai-Hrechynski village, Ternopil district), using a commercial portable water ozonator based on electrolytic ozone generation (ShenZhen BoRun Electronics Co., Ltd., Shenzhen, China). For each experiment, 500 mL of water was used, in accordance with the manufacturer's recommended volume for this device. Ozone generation was performed at room temperature (21 ± 1°C).

The mineral content of the water samples was assessed by measuring total dissolved solids (TDS) using a HI98301 conductivity meter (Hanna Instruments, Smithfield, Rhode Island, USA). Before measurement, the electrodes were rinsed with distilled water at room temperature and then immersed in the test sample. The pH of the water samples was determined using a 913 pH meter (Metrohm AG, Herisau, Switzerland).

The concentration of dissolved ozone was quantified using a PoolLab 1.0 photometer (Water-i.d., Eggenstein, Germany), which detects changes in color resulting from the reaction between ozone and N, N-diethyl-p-phenylenediamine sulfate. The measurement was performed photometrically by comparing the light absorbance of the reacted sample with that of an untreated control. Absorbance was assessed at specific wavelengths (530 nm and 620 nm), with the device utilizing internal calibration data to calculate ozone concentrations with the detection range of 0–4 mg/L. Tablet-form reagents were used during the measurement procedure.

To evaluate the efficiency of electrolytic ozone generation, ozone concentration was measured in real time over a 15-minute period. Measurements were taken every minute by withdrawing 10 mL of water from the container and immediately analyzing it photometrically for ozone concentration as previously described.

To assess the decomposition of ozone in the presence of bacteria, 9 ml of freshly ozonated water was mixed with 1 ml of *S. aureus* suspension (~8 log CFU/ml). After mixing, the samples were centrifuged at 3000 rpm for 3 minutes to separate bacterial cells, and the ozone concentration was measured in the resulting supernatant. Identical measurements were performed in parallel using sterile distilled water instead of the bacterial suspension as a control. In both cases, measurements were taken every 5 minutes during the first 30 minutes, followed by 10-minute intervals over the next 30 minutes. The first time point was recorded immediately after mixing (designated as 0 minutes in the graph), although due to centrifugation and handling, the actual measurement occurred approximately 6 minutes after sample preparation.

All experiments were conducted in triplicate. Means were compared using the Student's t-test. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION. Ozone concentration increased progressively during the 15-minute electrolysis period in both tap and spring water samples. However, the generation was significantly more efficient in tap water, reaching 2.98 ± 0.59 mg/L, compared to 2.00 ± 0.42 mg/L in spring water ($p < 0.05$). This difference may be attributed to the significantly higher ($p < 0.01$) mineral content of tap water (TDS 446.7 ± 3.5 mg/L compared to 421.0 ± 3.6 mg/L). The pH values of the two water sources were similar (7.44 vs. 7.41). A combined graph illustrating ozone accumulation in both water types over time is presented in Figure 1.

The higher efficiency of ozone generation observed in tap water compared to spring water can be attributed to its higher mineralization, as reflected by the TDS values. Since electrolytic ozone production relies on ionic conductivity, the greater concentration of dissolved salts in tap water likely enhanced the electrolysis process, resulting in a higher yield of aqueous ozone. Another possible explanation for the lower ozone concentration observed in spring water is the presence of organic matter, including native microbial contamination. Previous research demonstrated that untreated spring water may

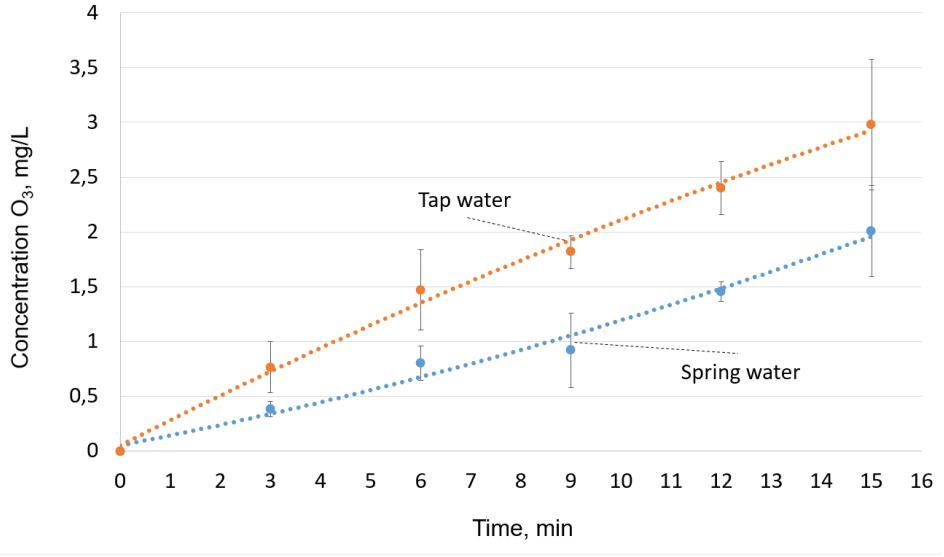

Fig. 1. Ozone concentration in tap and spring water during 15 minutes of electrolytic ozonation. Each point represents the mean ± standard deviation

contain up to ~2.7 log CFU/mL of microbial load [12]. Ozone is a highly reactive oxidant and readily reacts with organic compounds, including bacterial cells, extracellular polymers, and dissolved organic substances. These reactions not only accelerate ozone decomposition but may also reduce the efficiency of ozone accumulation during the generation process. In contrast, tap water, typically treated and filtered, contains fewer organic contaminants, potentially allowing more ozone to remain stable and detectable during generation. This is in line with the studies that have indicated that higher ionic strength, associated with increased TDS, can enhance the efficiency of electrolytic ozone generation [14], [15]. In traditional bubbling-based ozonation systems, it has been shown that higher mineral content in water can accelerate ozone decomposition, resulting in lower measured ozone concentrations. The authors stated that this effect is attributed to the catalytic role of dissolved ions and metal species, which promote ozone breakdown through complex radical-mediated mechanisms [16]. The influence of organic matter on ozone decomposition in ozonated water was investigated in the next experiment.

In clean water, the concentration of electrolytically generated aqueous ozone declined gradually over 60 minutes, starting at 3.98 mg/L and decreasing to 3.52 mg/L. When a suspension of *Staphylococcus aureus* was added, the initial ozone concentration was slightly lower at 3.88 mg/L and declined more rapidly, reaching 2.69 mg/L after 60 minutes. The effect of microbial presence was evident early in the experiment: by the 5-minute mark, the ozone concentration in the bacterial suspension had dropped to 3.71 mg/L, compared to 3.88 mg/L in clean water. After 20 minutes, the difference between the two samples widened to 0.62 mg/L, indicating accelerated ozone consumption in the presence of bacteria. This trend continued over time, with the largest difference of 0.83 mg/L observed at 60 minutes. These insights align with findings from previous studies. For instance, research has shown that dissolved organic matter can significantly accelerate ozone decomposition in water, reducing its stability and effectiveness as a disinfectant [9], [17], [18].

CONCLUSIONS. The higher efficiency of ozone generation observed in tap water compared to spring water is likely due to its greater mineral content and higher purity. The accelerated decomposition of ozone in the presence of *S. aureus* suggests that microbial cells and associated organic matter actively contribute to ozone consumption. This has important practical implications for optimizing ozone-based disinfection processes: real-world microbial loads may significantly reduce the effective contact time of active ozone in water. Understanding these dynamics is essential for adjusting ozone dosing and exposure strategies to maintain antimicrobial efficacy. Future studies will expand on this preliminary work by investigating a broader range of microbial species, varying biomass concentrations, and exploring the correlation between ozone decay and microbial inactivation efficiency.

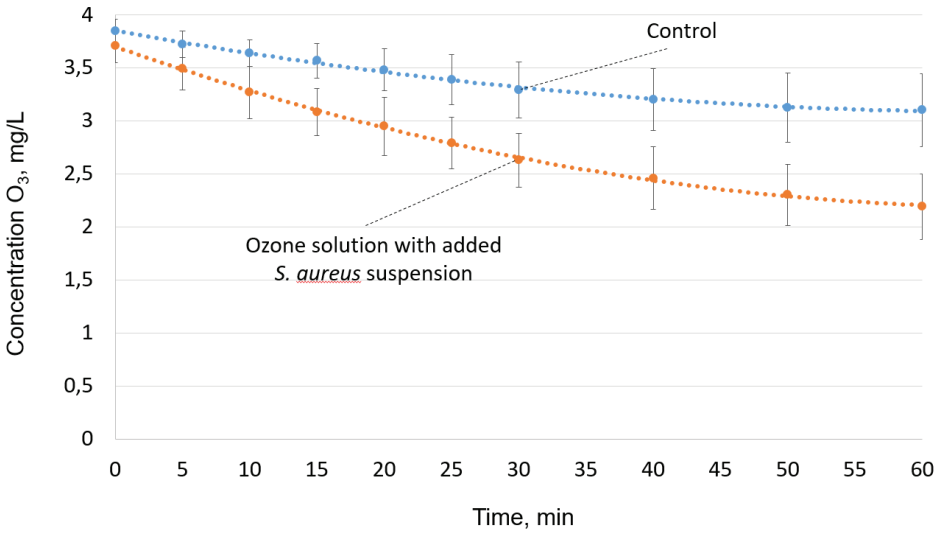

Fig. 2. Ozone decomposition rate in clean water and water mixed with *S. aureus* suspension. Each point represents the mean ± standard deviation

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[1] ТЕРНОПІЛЬСЬКИЙ НАЦІОНАЛЬНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ І. Я. ГОРБАЧЕВСЬКОГО МОЗ УКРАЇНИ
[2] НАЦІОНАЛЬНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ О. О. ОГОМОЛЬЦЯ

КІНЕТИКА ЕЛЕКТРОЛІТИЧНОГО УТВОРЕННЯ ТА РОЗКЛАДАННЯ ОЗОНУ У ПРІСНІЙ ВОДІ: ВПЛИВ БАКТЕРІАЛЬНОГО ЗАБРУДНЕННЯ ТА ДЖЕРЕЛА ВОДИ

Анотація

Вступ. *Мікробне забруднення води, зокрема, внаслідок скидання побутових стічних вод, становить серйозну загрозу громадському здоров'ю. Озон, як потужний окисник, розглядається як ефективна альтернатива традиційним дезінфекційним засобам. Останнім часом електролітичне озонування набуває популярності як безпечний та зручний метод одержання озонованої води без використання газоподібного озону.*

Мета дослідження *– оцінити ефективність генерації озону шляхом електролізу в різних типах води та дослідити кінетику його розкладу в стерильній воді та за наявності бактерій.*

Методи дослідження. *Озонована вода отримувалася електролітично з водопровідної та джерельної води. Концентрація озону вимірювалася в реальному часі протягом 15 хвилин. Стабільність озону вивчалася у стерильній воді та в присутності Staphylococcus aureus ATCC 6538 протягом 60 хвилин. Усі експерименти проводилися в трикратному повторенні. Статистичний аналіз здійснювався з використанням t-критерію Стьюдента.*

Результати й обговорення. *Концентрація озону була достовірно вищою у водопровідній воді (2,98 ± 0,59 мг/л), ніж у джерельній (2,00 ± 0,42 мг/л), що пов'язано з вищим вмістом мінералів, які покращують електроліз. У присутності бактерій спостерігалося прискорене зниження концентрації озону, що свідчить про його активний розпад у присутності мікроорганізмів та органічних речовин.*

Висновки. *Електролітичне озонування є ефективним методом одержання озонованої води, особливо в умовах підвищеної мінералізації. Водночас наявність мікроорганізмів значно пришвидшує розкладання озону, що слід враховувати у разі застосування озонованої води для знезараження.*

КЛЮЧОВІ СЛОВА: **водний озон; бактеріальне забруднення; електролітичне озонування; портативний озонатор; розкладання озону.**