



## Application of gaseous ozone and its aqueous solution for inactivation of pathogenic microorganisms: A literature review

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**Abstract.** Due to its potent oxidising properties, ozone presents itself as a promising disinfectant and may serve as a substitute for conventional techniques. The purpose of this study was to analyse the literature on the effectiveness of gaseous ozone and its aqueous solution against pathogenic and opportunistic microorganisms or their surrogates. More than 150 English-language sources have been studied, of which 72 have been selected for this review. To illustrate the effectiveness of ozone against bacteria, bacterial biofilms and viruses, data from selected publications are summarised in tables for the respective groups of disinfectant targets. In cases where data were presented in the form of kinetic curves in the reviewed publications, the maximum inactivation values were chosen. It was shown that both gaseous ozone and its aqueous solution are widely used due to their powerful bactericidal properties. The study noted that ozone is an effective oxidant that neutralises gram-negative, gram-positive, and spore-forming microorganisms, the biofilms they form, as well as fungi, protozoa, viruses, and prions. Among the industries that have implemented or may implement ozone applications are medicine (including dentistry and pharmacy), as well as the food industry and recreation. One of the notable advantages of using ozone is its relatively short half-life, with conversion to non-toxic oxygen. It was also noted that ozone treatment is non-thermal, which allows it to be used for processing fresh fruit and vegetables. The main disadvantages and challenges for safe implementation are ozone toxicity at exceeding the maximum permissible concentration in the air, corrosiveness, and explosiveness at high concentrations

**Keywords:** ozone; bactericidal properties; disinfection; non-thermal treatment; safety

### ✦ INTRODUCTION

Disinfectants play a crucial role in preventing the spread of infectious diseases and controlling microbial contamination. Scientific research helps determine the effectiveness of disinfectants in killing or inhibiting the growth of target microorganisms. Furthermore, the research helps assess the safety of disinfectants for human and animal health and the environment. Since the creation of the first commercial ozone generator, ozone has been used as an alternative to traditional disinfectants using chlorine-containing compounds [1]. The ability of ozone to inactivate a wide range of microorganisms has attracted interest among researchers. M. Murakami *et al.* [2] investigated the use of ozone in dentistry. Its effectiveness against various oral pathogens, including planktonic bacteria and biofilms formed by them, was emphasised. M. Marino *et al.* [3] inves-

tigated the effect of ozone on bacterial biofilms and found that ozone treatment can effectively disrupt the biofilm structure and reduce the viability of bacteria in it. M. Ziyaina & B. Rasco [4] demonstrated the wide possibilities of using ozone for the inactivation of microorganisms in the food industry. They have shown antibacterial effects of both gaseous and aqueous ozone against a wide range of microorganisms on dairy, meat products, and vegetables. M. Alimohammadi & M. Naderi [5] investigated the use of ozone and showed its effectiveness for inactivating viruses in indoor air. Researchers C. Wolf *et al.* [6] used ozone to inactivate enteroviruses in water.

As a strong oxidising agent, ozone effectively inactivates bacteria, damaging their cellular structure and disrupting vital metabolic processes. Among the means by

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which ozone can inactivate microorganisms are the following: oxidation of fatty acids and the breakdown of cell membranes, which results in increased permeability [7]; oxidation of amino acids and sulfhydryl groups in proteins and enzymes, which in turn leads to their denaturation and inactivation [8]; damage to nucleic acids [9]; and the generation of reactive oxygen species, which creates oxidative stress for microbial cells and viruses [10].

Until recently, Ukrainian science considered ozone mainly as a means of water purification. This is covered in the study by T.V. Hernaya [11], who among the methods of water disinfection described the use of ozone both independently and in combination with ultraviolet irradiation. O. Shmychkova *et al.* [12] demonstrated the use of ozone for the treatment of wastewater from pharmaceuticals. The complete destruction of antibiotic molecules by oxidation was shown. The purpose of this study was to review the English-language literature on the effectiveness of ozone against various groups of microorganisms and individual pathogens. This paper brings together a variety of scientific studies that both highlight the potential of ozone as a powerful disinfectant and reveal limitations and warnings regarding its use.

The Google Scholar web resource was used to search for literature. The literature was searched using the keywords “ozone generation”, “gaseous ozone”, “aqueous ozone”, “ozonated water”, “inactivation”, “log reduction” in combination “+” with groups of pathogens (“gram-negative bacteria”, “gram-positive bacteria”, “spore-forming bacteria”, “bacterial biofilms”, “fungi”, “protozoa”, “virus”, “prion”) and their individual species. English-language literature sources from the last 30 years were reviewed. Preference was given to papers describing ozone treatment alone, without the use of combined treatment with other non-thermal methods. Data on ozone-only treatment were selected

from publications with several types of treatment or combined treatment. Book chapters, dissertations, and reports or abstracts from scientific conferences were excluded from the search. Publications with the results of using ozonated oil were also excluded. The data from the selected publications were critically analysed, systematised, and presented in the form of a literature review. Both open-access articles and articles from paid resources were investigated.

#### ★ THE USE OF GASEOUS OZONE AND ITS AQUEOUS SOLUTION TO INACTIVATE BACTERIA

Due to the mechanisms described above, both gaseous and aqueous ozone have been effectively used to inactivate many Gram-negative microorganisms, including pathogens such as *Escherichia coli* [13, 14], *Salmonella* spp. [15], *Pseudomonas aeruginosa* [14, 16], *Legionella pneumophila* [17, 18], *Vibrio cholerae* [19, 20] and *Shigella* spp. [4, 19]. The effectiveness of ozone neutralisation of gram-positive flora, including *Staphylococcus aureus* [16, 21], *Streptococcus* spp., *Enterococcus* spp. [22, 23], and *Listeria* spp. [24]. Inactivation of gram-positive spore-forming microorganisms, both vegetative cells of *Bacillus* spp. [25], *Clostridium* spp. [26] and their spores [27, 28], was also reported. As various methods of treating varied contaminated substrates were employed, comparing the sensitivity of microorganisms to ozone based on diverse sources' outcomes is impractical. To illustrate the effectiveness of ozone against various microorganisms, data from selected publications are summarised in Table 1. In most of the selected cases, ozone-saturated water was used. In cases where data were presented in the form of kinetic curves in the reviewed publications, the maximum inactivation values expressed in logarithms of colony forming units (log CFU) per gram or millilitre of substrate were selected.

**Table 1.** Ozone inactivation of microorganisms

Pathogen	Inactivation (log CFU)	Processing time (min)	Ozone concentration (mg/L)	Temperature (°C)	Substrate	Processing type	Reference
<i>Bacillus cereus</i> (vegetative cells)	3.62	420	0.4	20±3	Cooked rice inoculated with bacterial suspension	Gaseous ozone	[26]
<i>Bacillus subtilis</i> (spores)	5.2	3	140	Not specified	Suspension of spores in a buffer solution	Ozone dissolved in a buffer solution	[28]
<i>Clostridium perfringens</i> – vegetative cells – spores	1.5 0.87	5	5	4 → 45 4 → 55	Microbial suspension applied to pieces of beef	Ozonated water (ozonation method not specified)*	[27]
<i>Enterococcus faecalis</i>	98%**	10	5 mg/min***	13	Naturally contaminated water from a private source	Water ozonated by bubbling	[22]
<i>Escherichia coli</i> O157:H7	2.9	30	1.5	10	Bacterial suspension applied to spinach leaves	Pressurised gaseous ozone 69 kPa*	[9]
<i>Legionella pneumophila</i>	>3	15	0.033****	22	Suspension of bacteria in 0.01 M phosphate buffer solution	Ozone dissolved in a buffer solution	[18]

Continued Table 1.

Pathogen	Inactivation (log CFU)	Processing time (min)	Ozone concentration (mg/L)	Temperature (°C)	Substrate	Processing type	Reference
<i>Listeria monocytogenes</i>	0.79	1	2-3	25	Pasteurised apple juice inoculated with a bacterial suspension	Passing gaseous ozone through juice samples	[24]
<i>Listeria monocytogenes</i>	3.7	10	50	Indoor (without specified values)	Bacterial suspension applied to TSA medium	Gaseous ozone	[25]
<i>Pseudomonas aeruginosa</i>	4.8	4	0.095****	Indoor (without specified values)	Suspension of bacteria in distilled water	Distilled water ozonated by bubbling	[16]
<i>Salmonella enterica</i> serovar <i>Enteritidis</i>	2.9	30	160	Not specified	Bacterial suspension applied to almonds	Pressurised gaseous ozone 12.5 psig***	[15]
<i>Shigella flexneri</i>	3.19 4.46	15 30	20.83 mg-min/l***	Indoor (without specified values)	Bacterial suspension in a wastewater model	Wastewater ozonated by bubbling	[19]
<i>Shigella sonnei</i>	5.6	1	2.2	Not specified	Suspension of bacteria in distilled water	Distilled water ozonated by bubbling	[4]
<i>Staphylococcus aureus</i>	5.4	2	0.095****	Indoor (without specified values)	Suspension of bacteria in distilled water	Distilled water ozonated by bubbling	[16]
<i>Staphylococcus aureus</i>	>2	4	2	23-24	Bacterial suspension applied to fabric (65% polyester, 35% cotton)	Gaseous ozone	[21]
<i>Streptococcus mutans</i>	2.91	10 sec*	Not measured, manufacturer's data are given: 0.052% (v/v), flow rate 13.33 ml/sec***	23	Bacterial suspension applied to glass beads	Gaseous ozone	[23]
<i>Vibrio cholerae</i>	3.26 4.52	15 30	20.83 mg-min/l***	Indoor (without specified values)	Bacterial suspension in a wastewater model	Wastewater ozonated by bubbling	[19]

**Notes:** \* – combined treatment with ozonated water for 5 min and subsequent heating of samples for 30 min was used; \*\* – units of measurement according to the original publication, corresponds to inactivation up to 2 log; \*\*\* – units of measurement according to the original publication; \*\*\*\* – initial ozone concentration

**Source:** developed by the author based on the results of a literary search

Thus, the effectiveness of both gaseous and aqueous ozone against a wide range of both Gram-negative and Gram-positive pathogens, including bacillus and clostridium spores, has been demonstrated.

#### ★ USE OF OZONE TO INACTIVATE FUNGI AND PROTOZOA

Ozone was used to inactivate fungal spores. Scientists have demonstrated the effectiveness of water ozonated by bubbling with a concentration of 2 mg/L at 20°C against *Aspergillus niger*, *Penicillium polonicum*, and *Trichoderma harzianum* [29]. Ozone gas at a concentration of 10 mg/L at 20°C for 15 minutes was used to inactivate *Candida albicans* and *Aspergillus fumigatus* on the surface of dense and porous materials. E.I. Epelle *et al.* [21] compared the effectiveness of ozonation with irradiation with ultraviolet light. The

authors attributed the higher efficiency of ozonation to the better permeability of gaseous ozone to porous materials, regardless of the orientation of the substrate relative to the source of the disinfectant. The same authors showed that under the same conditions, they could achieve better inactivation of bacterial strains of *Escherichia coli* and *Staphylococcus aureus*, suggesting that the thicker and stronger cell wall of fungi, compared to bacteria, makes it difficult for ozone to penetrate (and subsequently oxidise cell components). Even if fungi are not completely destroyed, ozonation slows their growth. Thus, O.J. Sujayasree *et al.* [30] proved that when citrus fruits artificially inoculated with *Penicillium digitatum* and *Penicillium italicum* cultures were treated with gaseous ozone at a concentration of 0.3-1 mg/L at 20°C for 4-9 weeks, the development of mould on fruits occurred a week later compared to the control. The same

treatment slowed or stopped the sporulation of fungi without any noticeable phytotoxicity to the fruit. Ozonation can neutralise not only fungal spores or vegetative cells, but also mycotoxins. Scientists have investigated the use of gaseous and aqueous ozone for the degradation of several mycotoxins (aflatoxins, ochratoxin A, fumonisins, deoxynivalenol, zearalenone, patulin) in various food products [31].

There is scarce data on the successful use of ozone against protozoa, as the cyst membranes make them resistant to disinfectants. For instance, K.E. Wainwright *et al.* [32] and A. Dumètre *et al.* [33] demonstrated that ozonation failed to inactivate *Toxoplasma gondii* oocysts at an ozone concentration of 6 mg/L for 12 minutes and 9.4 mg×min/L. However, ozone can still be used to inactivate some protozoa of medical importance. The results of studies by Z. Ran *et al.* [34] showed that ozonated water with an ozone concentration of 0.5 to 5 mg/L neutralised *Cryptosporidium* oocysts within 5 minutes. A more recent study by A.P. Ogura *et al.* [35] showed the killing of 3.44 log *Cryptosporidium parvum* by ozonated water with an ozone concentration of 10 mg/L for 5 minutes at 25°C. Ozone at a concentration of 5 mg/L was used in an attempt to inactivate *Giardia duodenalis* cysts in drinking water. The results of L.Y. Kondo Nakada *et al.* [36] showed that although this ozone concentration was not sufficient to inactivate all cysts, ozonation reduced the ability of cysts to cause disease in laboratory

animals and also reduced the reactivity of natural organic matter in treated water samples.

In general, the reviewed literature on the inactivation of fungi and protozoa showed moderate effectiveness of ozone against these microorganisms. This may be explained by the fact that eukaryotic cells have a more complex structure and are more resistant to ozone due to their thicker cell wall and the ability to form cysts that are quite resistant in the environment.

#### ★ AN ANALYSIS OF PAPERS ON THE USE OF OZONE TO INACTIVATE VIRUSES

Ozone has been used to inactivate viruses for several decades. Since the first studies of the antiviral properties of ozone, its ability to inactivate a wide range of human pathogenic viruses that cause both intestinal [6] and respiratory infections [5], as well as their surrogates [37], have been investigated and used. With the onset of the pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) virus, interest in the antiviral effect of ozone has increased considerably [5]. The data on virus inactivation by ozone are presented in Table 2. The inactivation values were expressed as logarithms of plaque forming units per millilitre of substrate (log PFU/ml) and/or the presence of the virus after treatment was confirmed by polymerase chain reaction.

**Table 2.** Inactivation of viruses with ozone

Pathogen	Inactivation (log PFU/ml)	Processing time (min)	Ozone concentration (mg/L)	Temperature (°C)	Substrate	Processing type	Reference
Adenovirus type 40	3.55	10	0.3	5	Phosphate buffer solution	Distilled water ozonated by bubbling	[38]
Hepatitis A virus	2.9 1.5	10	6.25	20	Virus applied to the surface of green onions Virus internalised in green onions	Distilled water ozonated by bubbling	[39]
Influenza virus type A subtype H1N1	99.999%*	150	20	23-29	Virus suspension dried on the surface of glass and plastic petri dishes	Gaseous ozone	[40]
SARS-CoV-2 coronavirus	3.3	55	6	25	Virus suspension dried on the surface of a stainless steel plate	Gaseous ozone	[41]
Mouse coronavirus (SARS virus surrogate)	3.0	60	20-25**	20	Virus suspension dried on the surface of a glass slide	Gaseous ozone	[42]
Mouse norovirus 1 (human norovirus surrogate)	4.1	10	6%***	25	Phosphate buffer solution	Gaseous ozone	[37]
Human norovirus	2.5	30	6.25	22	Alfalfa seeds inoculated with viral filtrate	Ozonated water (ozonation method not specified)	[43]
Poliovirus type 1	2.76	0.7 sec****	0.25	5	Phosphate buffer solution	Phosphate buffer solution ozonated by bubbling	[44]

Continued Table 2.

Pathogen	Inactivation (log PFU/ml)	Processing time (min)	Ozone concentration (mg/L)	Temperature (°C)	Substrate	Processing type	Reference
Respiratory syncytial virus A2	4.0	40	20	24	Virus on the surface of the N95 respirator	Gaseous ozone	[45]
Rhinovirus 1A and 14	2.0	60	28**	20	The virus is applied to a sterile glass surface	Gaseous ozone	[42]
Rotavirus strain Wa	8.0	1	25	25	Virus suspension	Distilled water ozonated by bubbling	[46]

**Notes:** \* – units according to the original publication, corresponds to inactivation of 5 log; \*\* – gradual increase of the concentration to the specified within 15 minutes; \*\*\* – units according to the original publication, corresponds to 60,000 mg/L; \*\*\*\* – units according to the original publication

**Source:** developed by the author based on the results of a literary search

The publications from the late 1980s and early 1990s reported data on effective inactivation (2.7-5 log) of hepatitis A virus by ozone (not included in this review). For the experiments, an aqueous ozone solution with a concentration of 0.25-1 mg/L was used, and the substrate was a virus suspension in phosphate buffer solution [47]. However, more recent publications have demonstrated both the effectiveness of aqueous ozone against hepatitis A virus applied to green onions (Table 2) and the inability of its gas state at a concentration of 5 mg/L to achieve a significant reduction in the concentration (<0.6 log) of hepatitis A virus on raspberry fruit during treatment for 3 minutes [48]. In this context, continuing research into the inactivation of viruses by ozone is important for optimising and improving the effectiveness of disinfection methods, ensuring the purity of water and food, and possibly preventing viral disease outbreaks.

#### ★ THE USE OF GASEOUS OZONE AND ITS AQUEOUS SOLUTION TO INACTIVATE PRIONS

While ozone has been widely investigated for its disinfectant properties against a wide range of microorganisms, including bacteria, viruses, and fungi, there are fewer publications on its effectiveness against prions. This is quite natural, given that these pathogens cause neurodegenerative disorders that are quite rare and that the research to inactivate these pathogenic proteins is difficult and expensive. The number of altered proteins was determined by Western blot, and qualitative changes were observed during the development of the disease in laboratory animals [49]. Prions are persistent in the environment and resistant to conventional disinfection methods such as heat treatment, chemical disinfectants, and ultraviolet radiation, which makes them difficult to inactivate [49]. Effective decontamination of prions is possible by hydrolysis of these proteins or destruction of the tertiary structure of the protein [50]. The study by N. Ding *et al.* [51] demonstrated the use of ozone as a disinfectant against prions. It has been shown that ozone dissolved in water by bubbling at a concentration of 13.7 mg/L can be effectively used to disinfect PrP<sup>Sc</sup>-contaminated clean water and wastewater at a treatment time of 5 min. In another publication, the

same researchers continued to demonstrate the effectiveness of ozone for wastewater treatment. They showed that inactivation of 2.8 log was achieved by ozonation with an initial concentration of 43.4 mg/L for 5 minutes, while preliminary settling of wastewater with the removal of some organic matter allowed to increase the inactivation of prion protein scrapie (PrP<sup>Sc</sup>) to more than 4 log [49]. The effectiveness of gaseous ozone has also been investigated for the inactivation of prions, specifically on the surface of stainless steel. It was found that prion disease developed later in mice intracerebrally implanted with prion-contaminated stainless-steel wires treated with gaseous ozone (25,000 mg/L, for 3 minutes) than in the control group of mice, indicating that ozone treatment reduces the infectivity of prions on stainless steel [50].

Although ozone treatment of prions has shown promise in laboratory studies, its practical application to neutralise these pathogenic proteins is still being explored. Research into the inactivation of prions by ozone can be challenging due to the unique properties of prions, but the potential benefits to public health safety make this area of research valuable.

#### ★ EFFICIENCY OF OZONE APPLICATION AGAINST BACTERIAL BIOFILMS

Bacteria can form biofilms on various surfaces of the human body, including teeth, mucous membranes, respiratory and urinary tracts, as well as on medical devices such as catheters, contact lenses and medical implants [2, 52]. Biofilms can be used to cover various artificial surfaces, including pipes of water supply and sewage systems, ventilation systems, and various equipment in such industries as the medical (including dentistry), pharmaceutical, food, and recreational sectors [2, 53]. Biofilms create a protective barrier for bacteria that can prevent the penetration of various disinfectants [2, 54]. Although ozone has demonstrated some effectiveness against biofilms, its ability to penetrate and inactivate bacteria within biofilms is generally lower than its effectiveness against planktonic bacteria [2, 25]. However, ozone can still have a certain effect on biofilms. Ozone treatment can disrupt biofilm structure, damage the matrix of extracellular

polymers, and reduce the total biomass of the biofilm. This can make the biofilm more sensitive to subsequent antimicrobial treatments and facilitate the removal of

bacteria from surfaces [2, 55]. To illustrate the effectiveness of ozone against biofilms, data from selected publications are summarised in Table 3.

**Table 3.** Effect of ozone on biofilms formed by bacteria

Pathogen	Surface type	Processing type	Ozone concentration (mg/L)	Processing time (min)	Effect	Reference
<i>Bacillus cereus</i>	Polypropylene plate	Gaseous ozone	45 ± 2	30	Inactivation 2.16 log CFU/cm <sup>2</sup>	[53]
<i>Bacillus subtilis</i>	Stainless steel plate	Gaseous ozone	1.4	4.5	Inactivation > 6 log CFU/ml	[54]
<i>Listeria monocytogenes</i>	Stainless steel cylinder	Tap water ozonated by electrolytic treatment	0.5	20	Inactivation 1.65 log CFU/cm <sup>2</sup> at static treatment (soaking) Inactivation 3.29 log CFU/cm <sup>2</sup> when treated with water flow (1 l/min)	[3]
<i>Listeria monocytogenes</i>	Stainless steel cylinder	Gaseous ozone	0.2 2	60 2	Inactivation 2.12 log CFU/cm <sup>2</sup> Inactivation > 5.33 log CFU/cm <sup>2</sup> (no growth)	[3]
<i>Pseudomonas aeruginosa</i>	Contact lens container (material not specified)	Distilled water ozonated by bubbling	4	20	Inactivation 7.42 log CFU/cm <sup>2</sup>	[52]
<i>Pseudomonas fluorescens</i>	Stainless steel cylinder	Tap water ozonated by electrolytic treatment	0.5	20	Inactivation 1.56 log CFU/cm <sup>2</sup> at static treatment (soaking) Inactivation 3.52 log CFU/cm <sup>2</sup> when treated with water flow (1 l/min)	[3]
<i>Pseudomonas fluorescens</i>	Stainless steel cylinder	Gaseous ozone	20	60	Inactivation 5.51 log CFU/cm <sup>2</sup>	[3]
<i>Salmonella</i> spp.*	Stainless steel plate	Ozonated water (ozonation method not specified)	16	20	Inactivation < 0.8 log CFU/cm <sup>2</sup> but potentiation (up to 2 log CFU/cm <sup>2</sup> ) was observed in combination with ultrasound (40 kHz)	[55]
<i>Staphylococcus aureus</i>	Stainless steel cylinder	Tap water ozonated by electrolytic treatment	0.5	20	Inactivation 2.15 log CFU/cm <sup>2</sup> at static treatment (soaking) Inactivation 4.47 log CFU/cm <sup>2</sup> when treated with water flow (1 l/min)	[3]
<i>Staphylococcus aureus</i>	Stainless steel cylinder	Gaseous ozone	20	20	Inactivation > 5.47 log CFU/cm <sup>2</sup> (no growth)	[53]

**Notes:** \* – a mixture of strains of *Salmonella enterica* serovar *Agona*, *Salmonella enterica* serovar *Enteritidis*, *Salmonella enterica* serovar *Infantis*, *Salmonella enterica* serovar *Typhimurium*

**Source:** developed by the author based on the results of a literary search

In general, further research in this area is promising and may lead to the discovery of new approaches or combinations of treatment methods that will increase the efficiency of destroying biofilms or preventing their formation on surfaces.

#### ★ OZONE PROPERTIES AND CHALLENGES ASSOCIATED WITH ITS USE

Ozone is a powerful oxidant and therefore a promising disinfectant due to its high reactivity and ability to penetrate deeper than liquid disinfectants [56]. This gas is unstable in the environment, it spontaneously decomposes to non-toxic oxygen with a half-life of about 30

minutes in aqueous solution and from 40 minutes to 25 hours in air, depending on the conditions (temperature, humidity, air movement) [57]. One of the substantial advantages of ozonation is its ability to operate without the need for thermal energy, which leads to energy savings. In contrast to conventional thermal treatment processes that rely on heating or boiling, ozonation uses the powerful oxidising properties of ozone to achieve the effect [58]. However, while ozonation itself saves energy, the energy requirement for ozone generation can vary depending on the method used [59]. Several methods can be used to produce gaseous ozone, among which the corona discharge method is most commonly

used for industrial ozonators [60]. In laboratories, the photochemical method can also be used to produce gaseous ozone [4]. Most often, an aqueous solution of ozone was obtained by bubbling gaseous ozone through water, which was saturated with it. Moreover, it can be produced by electrolytic method [61].

Ozone is widely used in various industries, but there are certain limitations to processing with this agent. Due to its strong oxidising properties, ozone not only destroys microorganisms but also damages everything it comes into contact with. Thus, there are limitations in the choice of an effective ozone dose and ozone treatment time. In medicine and dentistry, dose limitation is used to prevent tissue damage to patients. P.S. Subiksha [62], in the study on the use of ozone in dentistry, points out that even low concentrations of ozone can cause headaches, irritation or dryness of the nose, throat and eyes. There are also restrictions on ozone exposure for workers in other areas where it is used. In the commercial application of ozone, screening people who may come into contact with it in industry is mandatory because it affects the human respiratory tract. According to P. Wolkoff *et al.* [63], staying in a room with ozone in the air can cause eye irritation, while inhaling ozone can cause irritation and damage to the respiratory tract. People with pre-existing respiratory conditions, such as asthma or chronic obstructive pulmonary disease, may be more susceptible to the respiratory effects of ozone [64]. Its toxicity is primarily due to its strong oxidative properties. Exposure to high levels of ozone can lead to oxidative stress, inflammation, and damage to cells and tissues. According to C. Michaudel *et al.* [65], a single exposure to ozone caused rapid destruction of the epithelial barrier with increased neutrophil recruitment, reactive oxygen species production, airway hypersensitivity, and IL-33 expression in epithelial and myeloid cells in mice. In another *in vivo* experiment, G. Valacchi *et al.* [66] demonstrated that at concentrations of 0.8 mg/L for 6 days (exposure for 6 hours per day), ozone can affect both lungs and skin, causing depletion of  $\alpha$ -tocopherol and inducing stress-related reactions in both the skin epidermis and airway epithelium. Furthermore, M.I. Gilmour *et al.* [67] showed that ozone exposure increases the susceptibility of mice to pulmonary bacterial infection caused by *Streptococcus zooepidemicus*, reduces the function of alveolar macrophages, and increases the mortality of animals from infection.

In the food industry, the restriction applies to food processing, especially to fresh leafy vegetables. M.V. Shynkaryk *et al.* [56] demonstrated a change in the colour of spinach leaves after prolonged treatment with gaseous ozone at a concentration of 0.106 mg/L or short-term treatment with a higher (1.5 mg/L) concentration. Ozone is suitable for the treatment of rooms, surfaces, and equipment, but it should be borne in mind that the elements treated with ozone must be resistant to corrosion [68]. R. Pandiselvam *et al.* [69] showed that the use of ozone at a concentration of 47-106 mg/L for two months can lead to damage to rubber parts. Ozone decomposes to non-toxic oxygen, but when it reacts with a substrate, it can form by-products and modify the biomolecules in it. P. Li *et al.* [70] proved that ozone reacts with bromide

dissolved in water to form bromate, which is a carcinogen. T. Manasfi *et al.* [71] have demonstrated that ozonation by oxidation of polyunsaturated fatty acids produces aldehydes, ketones, and keto acids.

Another disadvantage is that ozone cannot be stored and decomposes quickly, and therefore on-site ozone production is mandatory. When producing ozone, it should be kept in mind that at elevated concentrations in the air, it may become explosive. The experiments conducted by K. Koike *et al.* [72] have shown that ozone mixed with oxygen at a concentration of more than 10-11% at room temperature and atmospheric pressure can lead to an explosive chain reaction until the complete decomposition of ozone. The above disadvantages and warnings do not detract from the benefits of ozone as an antibacterial agent. By overcoming these obstacles, it is possible to realise the potential benefits of ozonation in these industries, which would contribute to an improvement in hygiene, safety, and product and service quality. To mitigate the potential risks associated with ozone exposure, it is important to follow the recommended safety instructions and practices. Appropriate technical controls such as ventilation systems and personal protective equipment are recommended to minimise exposure and protect people from excessive ozone concentrations.

## ★ CONCLUSIONS

The literature review demonstrates the extensive application and efficacy of gaseous ozone and its aqueous solution in deactivating pathogenic microorganisms. The collected data from scientific publications demonstrate the bactericidal properties of ozone against various groups of microorganisms, including gram-negative and gram-positive bacteria, spore-forming microorganisms, biofilms formed by them, fungi, protozoa, viruses, and prions. This broad spectrum of action makes ozone a versatile option for microbial control in medicine and other industries. In most of the sources reviewed, ozone was used without combination with other treatments, but there are many publications demonstrating potentiation of inactivation when ozone and other thermal and non-thermal treatments are combined.

The introduction of ozone technology in sectors such as healthcare, food processing, and recreation is showing a growing recognition of its potential benefits. The relatively short half-life of ozone, which results in its conversion to non-toxic oxygen, is an advantage from a safety perspective. Furthermore, the non-thermal nature of ozone treatment allows it to be used in the processing of fresh fruit and vegetables, thereby improving product safety and quality. However, it is important to recognise the potential disadvantages and safety concerns associated with ozone. High concentrations of ozone are necessary to deactivate bacterial spores and protozoan oocysts, rendering the use of this treatment method challenging to deactivate these microorganisms on food or living organisms. Moreover, ozone toxicity at concentrations above the maximum permissible level in the air must be carefully regulated and monitored to ensure the well-being of workers in ozone-using industries. Additionally, the potential explosive nature of ozone in high concentrations requires proper handling and adherence to safety protocols.

Further research and development are needed to optimise the use of ozone in various industries and to address the challenges associated with its implementation. With proper precautions and new research, ozone technology can contribute to enhanced microbial control and improved safety standards in various sectors, ultimately benefiting public health and quality of life.

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#### ★ CONFLICT OF INTEREST

The author declares no conflict of interest.

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## Застосування газоподібного озону та його водного розчину для інактивації патогенних мікроорганізмів: огляд літератури

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**Анотація.** Завдяки своїм потужним окислювальним властивостям озон є перспективним засобом дезінфекції і може використовуватися, як альтернатива традиційним засобам. Метою даної роботи було проведення аналізу літературних даних стосовно ефективності газоподібного озону та його водного розчину проти патогенних та умовно патогенних мікроорганізмів чи їх сурогатів. Опрацьовано більше 150 англомовних джерел, з яких 72 вибрано для даного огляду. Для ілюстрації ефективності озону проти бактерій, бактеріальних біоплівки та вірусів дані з вибраних публікацій зібрано у таблицях по відповідних групах мішеней дії дезінфектанта. У випадках представлення даних у оглянутих публікаціях у вигляді кінетичних кривих обиралися максимальні значення інактивації. Показано, що як газоподібний озон, так і його водний розчин знайшли широке застосування завдяки їх потужним бактерицидним властивостям. Зазначається, що озон є ефективним окисником, який знешкоджує грам-негативні, грам-позитивні та спороутворюючі мікроорганізми, утворені ними біоплівки, а також грибки, найпростіші, віруси та пріони. Серед галузей, які впровадили чи можуть впровадити застосування озону є медицина (включаючи стоматологію та фармацію), а також харчова промисловість та рекреаційна галузь. Однією з помітних переваг застосування озону є його відносно короткий період напіврозпаду з перетворенням у нетоксичний кисень. Також зазначається, що обробка озоном є нетермічною і дозволяє його застосовувати для обробки свіжих фруктів та овочів. Основними недоліками та проблемою для безпечного впровадження є токсичність озону при перевищенні гранично допустимої концентрації у повітрі, корозійна активність та вибухонебезпечність при високих концентраціях

**Ключові слова:** озон; бактерицидні властивості; дезінфекція; нетермічна обробка; безпека