

**EVALUATING LOG REDUCTION OF *ESCHERICHIA COLI* ON STAINLESS
STEEL USING OZONE AND CHLORINE DIOXIDE GAS**

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ABSTRACT

This study evaluated the inactivation of *Escherichia coli* ATCC 11229 strain AMC 198 inoculated onto stainless-steel coupons using ozone gas at different concentrations and relative humidity levels. It also compared ozone and chlorine dioxide gas at equivalent oxidative concentration. Treatments for ozone gas were performed at 15, 50, 75, 100, and 125 ppmv under relative humidity levels of $66.8 \pm 0.6\%$, $75.3 \pm 1.1\%$, $91.1 \pm 1.3\%$ for an exposure time of 30 minutes. Increasing ozone concentrations achieved a log reduction of 3.6 ± 0.8 using 125 ppmv at 91.1% relative humidity. Statistical results demonstrated that both relative humidity and ozone concentrations had an effect on the inactivation of *Escherichia coli* ATCC 11229 strain AMC 198. When ozone and chlorine dioxide were compared at equivalent oxidative concentrations, chlorine dioxide reached a log reduction of 4.7 ± 0.3 and 4.6 ± 0.6 for 30 ppmv and 70 ppmv, respectively, under a relative humidity of 91.1%. But under a relative humidity of $66.8 \pm 0.6\%$, ozone and chlorine dioxide achieved similar inactivation. The results confirm that both gases can achieve significant reductions in *E. coli*, ranging from 3.6 to 4.8 log.

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CHAPTER ONE

INTRODUCTION AND GENERAL INFORMATION

Relevance of Disinfection in Food Safety

Food safety is a critical concern in the food industry, with microbial contamination posing significant risks to human health and economic stability.

Disinfection procedures play an important role in controlling microbes during food processing, storage, and packaging. In the food industry, chemicals are constantly used for disinfection and sanitization of surfaces that come into contact with the product to prevent contamination, but there is a difference between disinfecting and sanitizing (Pfuntner, 2020).

Disinfectants are stronger and aimed at killing or inactivating harmful microbes, including bacteria, fungi, and viruses (though not necessarily spores), especially in healthcare (e.g., medical instruments) or general environments (e.g., households, swimming pools, and water purifiers). According to the EPA in Product Performance Test Guidelines OCSPP 810.2200, a disinfectant must achieve at least a 6 log (99.9999%) reduction on both non-food contact and food contact surfaces with a minimum contact time of 10 minutes (Environmental Protection Agency [EPA], 2025; EPA & Office of Chemical Safety and Pollution Prevention, 2018). Sanitizers, on the other hand, are used to reduce microbial levels to safe thresholds specified by public health codes or regulations, particularly important in food-related settings (e.g., food processing plants) ((EPA), 2025). Under the Product Performance Test Guidelines OCSPP 810.2300, sanitizers should reduce microbial loads by at least 3 log (99.9%) on non-food contact

surfaces within 5 minutes, or 5 log (99.999%) on food-contact surfaces within 30 seconds and unlike disinfectants, sanitizers are not required to inactivate fungi and virus (EPA, 2012). Some chemicals may function as both sanitizers and disinfectants, depending on their concentration and how they are applied (Pfuntner, 2020, 2020).

Different agents that can cause foodborne outbreaks include microorganisms such as bacteria, parasites, viruses, and fungi, as well as non-microbial toxins like mycotoxins. According to the Centers for Disease Control and Prevention (2025), in the last two years 16 different foodborne outbreaks have occurred. The three most common bacterial species that cause the outbreaks are *Salmonella enterica*, *Escherichia coli*, and *Listeria monocytogenes* (CDC, 2024; Thomas et al., 2024). According to the CDC (2024), *Salmonella enterica* illnesses originated from different foods, with over 75% being linked to chicken, fruits, seeded vegetables, pork, other produce, beef, and turkey. While over 85% of *Escherichia coli* O157 illnesses were tied to vegetable row crops and beef. At the same time, more than 75% of *Listeria monocytogenes* cases were associated with dairy products, vegetable row crops, and fruits, though *Listeria* estimates are less reliable due to its rarity. The diversity from the implicated food sources highlights the importance of implementing food safety measures throughout the supply chain.

Escherichia coli as a Foodborne Pathogen

Escherichia coli (*E. coli*) is a gram negative, facultative anaerobe bacterial species that has been predominantly non-pathogenic and cross-sectional studies have shown that this bacteria is most commonly found in the intestinal microbiome of more than 90% of adult humans (Martinson & Walk, 2020). These bacteria support the host by

synthesizing vitamin B2 (riboflavin), which is essential for cellular functions, and vitamin K2 (menaquinone), which contributes to bone metabolism (Al-Zyoued et al., 2019). *E. coli* strains are normally classified by unique combinations of surface antigens, somatic (O), flagellar (H), and capsular (K), as well as its unique set of virulence factors; more than 180 O-groups and over 50 H-groups have been identified leading to a potential existence of nearly 10,000 serotypes (Callejón et al., 2015; Fratamico et al., 2016). Although most serotypes are harmless and live symbiotically in the gut, *E. coli* has been discovered to be an opportunistic bacterium, and certain strains pose significant public health risks by causing diarrhea, infections in the urinary tract, sepsis, and meningitis (Foster-Nyarko & Pallen, 2022a; Thomas et al., 2024). Diarrheagenic *E. coli* is generally divided into seven distinct pathotypes: enterotoxigenic (ETEC), enteropathogenic (EPEC), Shiga toxin-producing (STEC), enteroaggregative (EAEC), enteroinvasive (EIEC), adherent-invasive (AIEC), and diffusely adherent *E. coli* (DAEC) (Rojas-Lopez et al., 2018).

A well-known strain of STEC frequently implicated in foodborne outbreaks is *E. coli* O157:H7 and it was responsible for 92% of cases documented between 1998 and 2013 (Bennett et al., 2018). *E. coli* O157:H7 is most commonly transmitted through contaminated ground beef, unpasteurized milk, fresh produce, contaminated water, and personal contact (Mayo Clinic, 2022). Contamination can occur during processing, harvesting, or through cross-contamination in kitchens and foodservice environments (e.g., when handling beef and other meat products, through contaminated surfaces and kitchen utensils) (World Health Organization [WHO], 2018). The global burden that the

diarrheagenic *E. coli* causes is substantial. STEC is estimated to cause around 2.8 million infections each year leading to 3,890 cases of hemolytic uremic syndrome (HUS) and approximately 230 deaths (Majowicz et al., 2014). Just in 2010, STEC led to 12,953 Disability Adjusted Life Years (DALYs), ETEC for 2,084,229 DALYs, and EPEC for 2,938,407 DALYs (World Health Organization, 2015); DALYs are a standard public health metric that combines the years of life lost due to premature death with the years lived with disability, it is calculated by adding years of life lost with years of life lived with a disability and one DALY represents one lost year of healthy life (Moini et al., 2020). In 2019, the European Union One Health Zoonoses Report revealed that STEC was the third most reported zoonoses in the European Union (“The European Union One Health 2019 Zoonoses Report,” 2021). Zoonoses are infectious diseases that can spread from animals to humans. The primary vectors for STEC are cattle, sheep, deer, dogs, cats, pigs, horses, rabbits, chickens, turkeys, and goats. It can be transmitted to humans through contaminated food (raw or undercooked meat and produce with animal feces), water, or direct contact with infected animals (Desforges, 2021; WHO, 2020). Contamination points remain a challenge at farming, processing, and household levels.

Escherichia coli has been a fundamental model organism primarily used in microbiology and genetics, being valued for its rapid reproduction, well-characterized genome, and adaptability in laboratory experiments. These characteristics can be reflected in the famous phrase, “Tout ce qui est vrai pour le Colibacille est vrai pour l'éléphant” (“All that is true of *E. coli* is also true of the elephant”) (Foster-Nyarko & Pallen, 2022b; Friedmann, 2004).

Overview of the Gases Used in this Study

Chlorine dioxide (ClO₂) and ozone are oxidizing agents used as disinfectants which have a high effectiveness against many pathogenic microorganisms such as *Escherichia coli* (Oesinghaus et al., 2024). This paper discusses the application of gaseous ozone and chlorine dioxide in the food industry, focusing on their characteristics, methods of production and measurement, evaluation of their effectiveness on nonporous surfaces, and the hazards associated with exposure. The primary goal of this research is to evaluate the effectiveness of ozone and chlorine dioxide against *E. coli* on stainless steel coupons under controlled conditions with the following objectives: 1) to determine how relative humidity influences the inactivation rate of ozone, 2) determine the effect of gaseous ozone concentration on the log reduction in *Escherichia coli* inoculated onto stainless steel for a 30-minute treatment, and 3) to compare the efficiency of ozone and chlorine dioxide against *E.coli* under equivalent treatment conditions based on oxidation capacity.

The hypothesis for this study is that higher relative humidity will enhance the microbial inactivation efficiency of ozone gas, resulting in greater log reductions; however, chlorine dioxide is expected to achieve greater microbial inactivation than ozone under similar conditions.

CHAPTER TWO

LITERATURE REVIEW

Ozone

Ozone is composed of molecules that contain three oxygen atoms (O_3), and its most outstanding feature is that it is a powerful oxidizing agent. It is a colorless gas that, at high concentrations, can appear pale blue. Although sometimes described as odorless, ozone actually has a sharp, pungent smell due to the combustion of oxygen being released with a scent similar to the one that appears after an electrical storm (Cristiano, 2020; Rubin, 2001).

Ozone has been demonstrated to have a high inactivation capacity against bacteria, viruses, protozoa and endospores, but the trioxygen molecule is unstable which is why the gas must be generated on site (Khadre et al., 2001).

Ozone is a versatile disinfectant that is used across different industries due to its oxidative and no residue properties. For instance, in water treatment ozone is extremely effective in inhibiting bacteria, viruses, and pathogens, ensuring safe drinking water and purifying industrial wastewater (SUEZ Water Technologies & Solutions, 2025). In addition, in the food industry ozone is utilized for sanitizing, washing, water treatment, odor removal, and in-plant disinfection of equipment, fruits, vegetables, and meat (Absolute Ozone, 2024b; Dubey et al., 2022). During pandemic of COVID-19 attention increased in the potential of ozone for decontamination and therapeutic applications, being widely used to disinfect surfaces, materials, and indoor environments while also showing a possible therapeutic effect (Epelle et al., 2023). Ozone therapy has been used

to treat arthritis, ischemic heart disease, macular degeneration, cancer, fighting viral diseases (i.e., HIV and SARS), wound disinfection, and boosting immune system. However, it is important to note that the U.S. Food and Drug Administration (FDA) issued warnings against the use of ozone therapy in medical treatments (mostly used as a topical disinfectant either as a gas or in water) because of the lack of evidence supporting its safety and effectiveness (Seymour & Biggers, 2023).

Table 1. Physical and Chemical Properties of Ozone

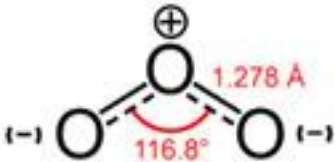
Property	Value	Reference
Molecular Formula	O ₃	(National Center for Biotechnology Information, 2025)
Molecular Structure		(Xue et al., 2023)
Color	Light blue	(Oxidation Technologies, 2024d)
Smell	Odor threshold ranges from 0.001 to 0.05 ppm. Ozone has a characteristic odor at concentrations below 2 ppm, the exposure can desensitize and at high it produces a pungent odor at high concentrations (	(National Center for Biotechnology Information, 2025; Oxidation Technologies, 2024d; Ozone Solutions, 2021a)
Aqueous Density	1.614 g/L at -195.4 °C	(National Center for Biotechnology Information, 2025)
Gaseous Density	2.144 g/L at 0 °C (1 atm); pure ozone	(Absolute Ozone, 2024a; Joseph et al., 2021)
Molecular Weight	48 g/mol	(Epelle et al., 2023)

Table 1. Continued

Vapor Density (Relative to Air (air = 1.0))	1.7- Heavier than air; will sink	(National Center for Biotechnology Information, 2025)
Vapor Pressure	41257 mmHg at 10.4 °F (>1 atm)	(National Center for Biotechnology Information, 2025)
Oxidation Potential (V)	2.07 V	(Oxidation Technologies, 2024c)
Melting Point	-192.5 ± 0.4 °C	(Joseph et al., 2021)
Boiling Point	-111.9 ± 0.3 °C	(Joseph et al., 2021)
Enthalpy of Formation	142.12 kJ/mol	(Absolute Ozone, 2024a)
Solubility in Water	1370 mg/l (1 atm)	(Absolute Ozone, 2024a)
Critical Temperature	-12.1°C	(Oxidation Technologies, 2024d)
Critical Pressure	54.5 atm	(Epelle et al., 2023)
Chemical Safety	 Oxidizer Corrosive Acute Toxic Irritant Health Hazard Environmental Hazard	(National Center for Biotechnology Information, 2025)

Prior to 1997, regulatory restrictions limited the commercial use of ozone in food processing, since the FDA required food additives to undergo approval under the Federal Food, Drug, and Cosmetic Act (FDCA) and even though ozone was granted the GRAS status (Generally Recognized as Safe) for disinfection of bottled water (maximum residual of 0.4 mg/L) in 1982, other food applications required separate Food Additive Petitions (FAPs), causing delays and withdrawals (Rice et al., 2002).

In 1997, an expert panel affirmed the safety of ozone as a food disinfectant, which lead to and industry-supported FAP that was formally approved by the Food and Drug Administration (FDA) as an “Antimicrobial Agent for the Treatment, Storage and

Processing of Foods in Gas and Aqueous Phases” in 2001. This document includes data on most applications for ozone in gaseous and aqueous phases on food (Rice et al., 2002; U.S Food and Drug Administration [FDA], 2024). In 2002 the U.S. Department of Agriculture’s Food Safety and Inspection Service (USDA/FSIS) authorized the use of ozone for direct contact with meat and poultry, including raw, freshly cooked, and pre-packaged products (Ozone Solutions, 2021b; Rice et al., 2002) There is no maximum set limit for allowable concentration for ozone, the range is determined under the Good Manufacturing Practices; meaning that the expected dose of ozone being applied should be the minimum effective dose that achieves microbial reduction without compromising either product quality or worker safety (Rice et al., 2002).

Background

Ozone gas was first identified in 1785 by the Dutch physicist Martinus Van Marum during his experiments with electrostatic machines. These machines released electrical discharges into the air, resulting in the generation of a gas which was characterized by a distinctive odor and a strong reactivity with mercury and describe it as the scent of electric matter (Carlos & Silvia, 2024; Schwartz, 2010). However, it was not until 1840 that the German chemist Christian Friedrich Schönbein formally synthesized the concept of ozone, advancing the understanding of this unknown gas (Schwartz, 2010). Schönbein isolated the gas responsible for the characteristic odor observed in electrolysis and named this gas as "ozone", which is derived from the Greek word “ozein” meaning "to smell", concluding that the odor was due to the tiny particles of oxidized electrode material formed from the reaction rather than the electricity itself

(Carlos & Silvia, 2024; Rubin, 2001; Stolarski, 2001). While researching at the University of Basel in Switzerland Schönbein announced the discovery of ozone through a letter addressed to the French Academy of Sciences titled "Investigation into the nature of the odor of certain chemical reactions" (Carlos & Silvia, 2024; Rubin, 2001; Stolarski, 2001) Although the molecular formula for ozone (O_3) was proposed in 1865 by Swiss chemist Jacques-Louis Soret and corroborated by Schonbein in 1867 (Carlos & Silvia, 2024; Rubin, 2002). Schönbein fell victim to a *Bacillus anthracis* infection while testing a new preservation method for meat and by the time of his death in 1868 ozone became an accepted concept in the chemistry world (Giuliani et al., 2018; Rubin, 2002).

In 1857, German electrical engineer Werner von Siemens developed an induction tube that was designed to produce ozone in reasonably large quantities, that used high-voltage electrodes, to explore the different uses ozone could have (Schwartz, 2010). Siemen's ozonizer design consisted of two co-axial glass tubes fused together with tin foil coatings on the inner and outer sides, being connected to the terminals of the induction coil that generated high-voltage current; a slow stream of pure, dry oxygen is passed through the space between the tubes, then subjected to an electrical discharge to generate ozone producing between 10-15% ozone in the oxygen, ideally in a low ambient temperature ($0^{\circ}C$) to prevent sparks from the discharge (Schwartz, 2010).

The first practical application of ozone was for water disinfection at the Oudtshoorn Water Treatment Plant in the Netherlands in 1893. By 1906 France had implemented this water treatment plan for it to be later followed by the United States in 1908 (Gray, 2013). Water treatment using ozone spread at fast speed across Europe in

1920. Presently ozone is a widely recognized disinfectant with over 3,000 municipal treatment facilities using it globally. Despite its powerful disinfectant properties, ozone is used less than chlorine for water treatment because chlorine is less expensive, easier to store, and provides a longer residual effect in water due to its greater stability (Gray, 2013).

Methods of Generation

Ozone generators work by transforming oxygen from different sources, for example from ambient air, dry air, or concentrated oxygen, into ozone (O_3). The generator splits the oxygen molecules (O_2) from stable atoms into unstable single atoms by applying an electrical charge to them and the single atoms bond with stable oxygen molecules resulting in ozone (Ozone Solutions, 2024).

Because of ozone's instability it is necessary to generate ozone in-site and on-demand. There are many different types of ozone generators, UV ozone generators, corona discharge ozone generators, and electrolytic ozone generators. UV and electrolytic ozone generators have limited ozone generation and are more difficult to control during production (Bocci, 2010).

Ultraviolet Ozone Generators

UV generators use ultraviolet radiation to convert ambient air into ozone gas. These generators typically use low-pressure mercury lamps that emit ultraviolet light, generally at 185 nm, to split the atmospheric oxygen molecules (O_2) into atomic oxygen (O). Then, the atoms recombine with the available O_2 to form ozone (O_3) (Inserv Company, 2025). This process goal mimics the natural ozone formation in the Earth's

stratosphere, where the solar UV light of similar wavelengths splits O_2 , leading in the end to ozone generation (Oxidation Technologies, 2024c).

Corona Discharge Ozone Generators

The most common ozone generation in both industrial and research settings use non-thermal plasma via corona discharge. In a corona discharge ozone generator, high-voltage electrical energy is applied across an air gap within a specially designed corona cell to split oxygen molecules (O_2) to form ozone (O_3). A dielectric barrier is located in the air gap to evenly distribute the electron flow, this increases the volume of oxygen that is exposed to the discharge and thereby improving the ozone production efficiency (Abby & Oxidation Technologies News, 2025; Andersen & Aubermann, 2023).

Electrolytic Ozone Generators

Electrolytic generators offer an advantage over UV and corona discharge generators by producing ozone directly in water, replacing the need of to generate the ozone and then dissolve it. In this system an electric current splits water molecules (H_2O) into hydrogen (H_2) and oxygen (O). The liberated oxygen atoms are then converted into ozone (O_3), but this requires isolating oxygen from hydrogen and applying an electrical charge to drive the reaction (Oxidation Technologies, 2024a). However, it is important to note that electrolytic ozone generators have a high energy consumption and are only reliable when using ultra-pure water.

Measurement and Units

Ozone gas sensors are generally divided into direct and indirect types. Direct sensors measure gases in real time but often require large, costly, and maintenance

intensive instruments. On the other hand, indirect sensors can detect gases through interactions with an active surface, allowing for a more portable, and cost effective device for field use (Petruci et al., 2022).

Direct methods include UV and MIR absorption spectroscopy, where ozone is measured by relying on the absorption of the molecules at wavelengths found in the Hartley band UV region of 254 nm and at the 8.65 μm in the mid-infrared. The loss of transmitted light is quantified using the Beer-Lambert law to determine the ozone concentration (Petruci et al., 2022). Photoacoustic spectroscopy is a technique that measures the sound waves that are generated when the gas absorbs light, but the sensitivity is limited (Spagnolo et al., 2023; Wojtas et al., 2014). Indirect measurement methods can include metal-oxide semiconductor-based sensors, amperometric sensors, and colorimetric tubes. Metal-oxide semiconductors detect ozone by measuring the changes that occur in the electrical conductivity of a heated metal oxide surface at the time the ozone molecules are interacting. This offers high sensitivity and low cost but is limited by the high operation temperatures (Korotcenkov & Cho, 2012). Amperometric sensors dissolve ozone gas into an electrolyte where it undergoes an electrochemical reaction in the working electrode that is held at a fixed potential. The resulting current is measured against a counter/reference electrode which is directly proportional to the gas concentration (Baron & Saffell, 2017; Knake et al., 2005). Another indirect method is the use of colorimetric tubes. These are glass tubes marked with a scale that is filled with chemical reagents that change color at the moment that it reacts with the gas. To take a measurement with colorimetric tubes, the tube is placed in a hand pump that draws a

known volume of air sample through it (Levitt, 2018). A reading of the ozone concentration is made based on the scale on the tube and the volume of air sample pulled through it.

Across different studies, the most commonly used units for reporting ozone concentration, whether it is in air or water, are milligrams per liter (mg/L), grams per cubic meter (g/m^3) and parts per million (ppm). However, it is very easy to misinterpret these units if the basis (e.g., mass, volume, molar) and the application (e.g., water, air, oxygen) is not clearly specified. Ozone concentration measurement involves different methods and units, which can complicate the comparison of sanitization studies. Also, possible unit conversion errors, and inaccurate calibration of ozone monitors, may lead to discrepancies when interpreting ozone effectiveness (Chawla et al., Microbial decontamination in the food industry: Novel methods and applications/2012). It is possible to express ozone concentration as mass per volume, mass per solvent mass, or mass per total mass (percent mass), though the mass-to-volume ratio is generally preferred due to its simplicity at the time of performing calculations and it reduces the risk of misinterpreting the results. For aqueous ozone, ppm_{mass} is most common, while $\text{ppm}_{\text{volume}}$ is used for low gaseous concentrations. Also, g/m^3 is standard for higher gaseous concentrations and this mass-to-volume ratio is recommended as it requires only the mixture's volume to calculate total ozone mass (Chawla et al., Microbial decontamination in the food industry: Novel methods and applications/2012). Percent mass should be used carefully, since it is normally labeled only as % which can be easily confused with percent by volume. This ambiguity is more problematic when using

gaseous ozone, since gases expand or contract with temperature and pressure, making volume-based units less reliable.

Applying accurate conversions between units is critical for research and practical applications. Below are the most common used conversion factors for ozone, derived from standard conditions and molar volume calculations (Chawla et al., Microbial decontamination in the food industry: Novel methods and applications/2012; Myers, 2023; Ozcon Environmental Solutions, 2025): In water: $1 \text{ g/m}^3 = 1 \text{ mg/L} = 1 \text{ ppm (w/w)}$;

In air: $1 \text{ g/m}^3 = 1 \text{ mg/L} = 467 \text{ ppmv}$ and $1 \text{ ppmv} = 2.14 \text{ mg/m}^3$; percent by volume: $100 \text{ g O}_3/\text{m}^3 \approx 6.99\% \text{ O}_3 \text{ by volume}$, $1\% \text{ O}_3 \text{ by volume} = 14.3 \text{ g O}_3/\text{m}^3$.

These conversions account for molar volume (22.4 L/mol), for ideal gases at standard temperature and pressure ($T = 298.15^\circ\text{K}$, $P = 1 \text{ atm}$), and ozone's molar mass (48 g/mol):

$$1 \text{ mg/L O}_3 = 509.79 \text{ ppmv O}_3: \left(\frac{1000 \text{ mg}}{\text{m}^3} \right) \left(\frac{24.47 \text{ L/mol}}{48 \text{ g/mol}} \right) \left(\frac{1 \text{ atm}}{1 \text{ atm}} \right) = 509.79 \text{ ppmv}$$

$$1 \text{ ppmv O}_3 = 1.96 \text{ mg / m}^3: (1 \text{ ppm}) \left(\frac{48 \text{ g/mol}}{24.47 \text{ L/mol}} \right) \left(\frac{1 \text{ atm}}{1 \text{ atm}} \right) = 1.96 \text{ mg/m}^3$$

Table 2. Standard Units for Ozone Measurement and Production in Air and Water.

Category	Unit	Description
Ozone in Air (Gas Phase)	Parts per million by volume (ppmv)	Volumetric measurement of ozone in air
	Milligrams per cubic meter (mg/m ³)	Mass-based ozone concentration in air
	Grams per cubic meter (g/m ³)	Industrial unit for ozone in air
	Milligrams per liter (mg/L)	
Ozone in Water (Liquid Phase)	Milligrams per liter (mg/L)	Most common unit for aqueous ozone concentration
	Parts per million (ppm)	Higher concentration applications
	Grams per liter (g/L)	
	Parts per billion (ppb)	Trace amounts of dissolved ozone

Table 2. Continued.

Ozone Production & Dosage	Grams per hour (g/h)	Ozone generator production rate
	Milligrams per liter per minute (mg/L/min)	Used in water treatment applications
	Pounds per day (lb/day)	Industrial ozone dosage unit

Ozone: Gaseous vs. Aqueous

There are two different application methods for ozone, gaseous and aqueous. While aqueous ozone has been established in industrial sanitization and disinfection, gaseous ozone is increasingly getting recognized for its ability to treat surfaces where aqueous applications are less practical (Qian et al., 2022).

Gaseous Ozone

Gaseous ozone is effective because of its ability to diffuse freely and reach irregular surfaces, hidden crevices, and even airborne microorganisms (Cristiano, 2020; Epelle, Macfarlane, Cusack, Burns, Amaeze, et al., 2022). This makes it attractive for the use of disinfecting enclosed spaces without leaving residue since ozone becomes ordinary oxygen. Since gaseous ozone leaves the substrate dry, it eliminates the need of additional drying after treatment. However, to disinfect or sanitize surfaces, it is necessary to use a cleaning step prior to treatment in order to remove any organic matter or debris (Epelle, Emmerson, et al., 2022). Concentrations in air can reach high levels (e.g., up to 50 ppmv) relatively fast, depending on the capacity of the generator used and the temperature. Ozone is the most stable at 20°C for approximately 30 to 40 minutes before breaking down, when temperatures rise to 40°C the half-life start to decrease at 15 minutes, at

60°C it decreases at three to four minutes, and when temperatures rises above 70°C ozone's decomposition can occur under one minute (Epelle, Emmerson, et al., 2022; Pinnacle Ozone Solutions, 2025b).

Aqueous Ozone

Ozone concentration in water is limited by how much it can dissolve and how quickly it can be transferred, which reduces its effectiveness. Aqueous ozone simultaneously cleans and disinfects, and having surfactants can improve the stability in solution (Epelle, Emmerson, et al., 2022). It is generated by dissolving ozone into water, which produce a reactive solution that is capable of direct contact sanitization. This method is widely used in the food and medical industries; however, it wets the treated substrate requiring a drying step afterward, an especially important consideration for porous surfaces (Epelle, Emmerson, et al., 2022). Factors such as pH, temperature, and water composition have an influence in the stability of ozone. Ozone is more stable at low temperatures and under low pH values (acidic = pH levels below five) conditions, while higher pH values (alkaline= pH levels above eight) accelerate decomposition. Under acidic conditions (pH levels below five) ozone decomposes between 10-30 minutes, but at neutral conditions (pH levels between six and seven), the decomposition rate starts to increase, especially if a catalyst (e.g., 3% Pd/SiO₂ refers to a palladium (Pd) catalyst on a silica (SiO₂) support, containing three percent palladium by weight) is present (Lin et al., 2002; Pinnacle Ozone Solutions, 2025a). In contrast, at alkaline levels (pH >8) ozone becomes highly unstable and decomposes under one minute (Pinnacle Ozone Solutions, 2025a). Aqueous ozone's stability can also be dependent on the

temperature of the water. As the water gets warmer, the concentration of ozone it can retain decreases from about 0.64 mg/L at 5°C to 0.29 mg/L at 35°C (Pinnacle Ozone Solutions, 2025b). As a result, continuous generation and careful process are required to maintain levels adequate for antimicrobial disinfection (Gunten, 2003).

Both gaseous and aqueous ozone are highly effective disinfectants, but their performance is influenced by factors as pH (for aqueous), temperature, and relative humidity (for gaseous), which can help with stability and efficacy (Dubuis et al., 2020; Gunten, 2003). Consequently, the choice between these two methods ultimately will depend on the context of the application, with aqueous ozone being favored for direct contact sanitization (e.g., water treatment) and gaseous ozone is most used for surfaces and air treatment (e.g., disinfection of medical equipment) (Epelle et al., 2023; Hamid et al., 2024).

Factors Influencing Ozone Efficiency

When using ozone gas, it is important to consider the different intrinsic and extrinsic factors that can affect antimicrobial efficiency. Some intrinsic factors include the inoculation concentration and volume, type of microorganism and the physiological state of the microorganisms are in, surface that is used for inoculation and the extrinsic factors are humidity, temperature, gas concentration and exposure time, and uniformity inside the treatment chamber (Hamid et al., 2025).

The inoculation concentration and volume are important because, to properly assess antimicrobial efficacy, there should be an initial microbial load of at least three to six log on the inoculated surface (Hamid et al., 2025; Newbern, 2021). Additionally,

selecting an appropriate inoculum volume is essential. Volumes greater than 100 µl can result in the formation of bacterial lawn (Epelle, Macfarlane, Cusack, Burns, Thissera, et al., 2022). A bacterial lawn is dense and uniform layer of bacteria growth medium across the surface of a solid medium, typically resulting from spreading high inoculum volumes (Epelle, Macfarlane, Cusack, Burns, Thissera, et al., 2022; Mou et al., 2019). These plates are often used to determine bacteria sensitivity against antimicrobial agents such as, antibiotics, disinfectants, mouthwashes, and natural antimicrobials (e.g., garlic) (Microbiology Society, 2016; Royal Society of Biology, 2019).

The type of microorganism and their physiological state also play a significant role in determining the susceptibility to ozone. Different microbial species have different sensitivities to the disinfection of gaseous ozone, for instance, gram negative bacteria generally show higher sensitivity when compared to the gram-positive bacteria; this is due to the cell wall structure (Broadwater et al., 1973; Khadre et al., 2001; Marino et al., 2018). The physiological state of the cells also influences their response to gaseous ozone, microorganisms that are in their exponential phase tend to be more vulnerable since they have not developed stress response mechanisms. Whereas when they are in the stationary phase cells have a greater tolerance due to the stress-induced activation of protective defenses (Guzel-Seydim et al., 2004; Restaino et al., 1995).

Another intrinsic factor is the type of surface used for the inoculation. Smooth, non-porous surfaces (e.g., stainless steel, glass, ceramic) allow ozone to have a greater contact and improve microbial inactivation when compared to porous, rough surfaces (e.g., fabric, wood, rubber) since the creases can shield the cells (Khadre et al., 2001;

Moore et al., 2000). Not only that, but if a surface is hydrophobic it may reduce the ability of ozone to penetrate while a hydrophilic surface can manage to obtain a more uniform contact of ozone (Hudson et al., 2007).

Increasing relative humidity enhances the antimicrobial effectiveness of gaseous ozone, thus shortening the exposure times needed for effective disinfection (Pascual et al., 2007). This effect is believed to be the result of the hydration of microbial cells, since this promotes the membrane permeability which makes the cells more vulnerable and as relative humidity increases, ozone forms additional reactive oxygen species such as hydroxyl radicals (OH) and superoxide anions (O_2^-) (Ishizaki et al., 1986).

Temperature is another extrinsic factor that influences ozone. When temperature increases the relative humidity lowers, which can reduce the antimicrobial activity of ozone (Bencloski, 1982). In some studies it has been reported that elevated temperatures (30 – 45 °C) enhance virus or bacterial inactivation, but the effects are often linked to the increases in water vapor or faster chemical reactions rather than the temperature alone (Blanchard et al., 2020; Thill & Spaltenstein, 2020). Higher temperatures decompose ozone more rapidly, the decomposition produces free radicals that can alter ozone's disinfection mechanism, while at lower temperatures ozone becomes more stable though higher concentrations may be needed to achieve complete sterilization (Epelle, Emmerson, et al., 2022; Hamid et al., 2025). Another research showed no impact of temperatures ranging from 16 – 25°C on microbial inactivation (Pironti et al., 2021), this suggests that the exposure time is more critical.

Gas concentration and exposure time is often expressed as CT values (concentration x time). Generally, having higher ozone concentrations or longer exposure times result in a high microbial inactivation, though this relationship may not always be linear, as factors as microbial resistance (how sensitive the bacteria is to gaseous ozone treatment) and interactions with the surface can lead to reduced effectiveness (Khadre et al., 2001; J. G. Kim et al., 1999). While, low concentrations when applied for extended period can achieve similar inactivation levels as high concentrations applied for short periods, all will depend on the microorganism and environmental conditions (Restaino et al., 1995).

The uniformity inside the ozone treatment chamber, ozone and humidity distribution, may affect the inactivation outcome. This is because poor circulation can lead to uneven exposure, usually fans are used to circulate everything inside the chamber (Khadre et al., 2001; Tizaoui et al., 2022). Ventilation, chamber size, and the placement of the samples being treated can impact the ozone circulation and lead to pockets where the gas does not reach. Studies have shown that the use of controlled air circulation systems or using multiple injection points can improve the uniformity inside the chamber, this ensures that the inoculated surfaces receive a uniform treatment (Epelle et al., 2023; Hudson et al., 2007). Fans and airflow systems play a crucial role, as proper circulation, monitoring, and modeling help prevent uneven sample exposure, particularly in industrial and medical settings (Hamid et al., 2025; Tamaddon Jahromi et al., 2022). Without proper uniformity, the efficacy of the assessment may be limited.

Risks of Exposure and Safety Limits

The most common symptoms of exposure to low ozone concentrations (0.1–0.3 ppmv) are headaches, coughing, dry throat, shortness of breath, a heavy feeling in the chest, and fluid in the lungs; whereas exposure to high concentrations (>5 ppmv) can cause more severe symptoms, such as asthma and other serious respiratory effects (The National Institute for Occupational Safety and Health [NIOSH], 2019).

According to the California Air Resources Board (2025) ozone generators used for air purifiers can cause damage to the cells in the lungs and respiratory airways, prolonged use of air purifier's may cause irritation and inflame the lining of the respiratory system, leading to coughing, chest tightness, shortness of breath, and impaired breathing. Ozone exposure can also worsen asthma symptoms and further deteriorate the health of individuals who are already in poor condition. Populations at higher risk include children, the elderly, and people with weakened immune systems. Because of these health concerns, California has banned the sale of indoor ozone generators that produce ozone emissions above 0.050 ppm, ensuring that air cleaning devices sold in the state meet strict safety standards (CARB, 2025).

The Occupational Safety and Health Administration (OSHA) states that workers should not be exposed to concentrations higher than 0.10 ppm for an eight-hour period and they established a 15-minute short term exposure limit (STEL) of 0.3 ppm for ozone. The STEL was determined on observations of how it can decline pulmonary function from excessive exposure (CDC & NIOSH, 2011). A study from Kleinfeld et al. (1957)

showed that welders developed pulmonary edema after being exposed to ozone concentrations of 9 ppm and other air pollutants (CDC, 1994).

The National Institute of Occupational Safety and Health (NIOSH) guidelines limit workers to have an exposure of ozone not to exceed 0.10 ppm at any time and the American Conference of Governmental Industrial Hygienists (ACGIH) recommends limits depending on the type of work, for heavy work 0.05 ppm, for moderate work 0.08 ppm, for light work 0.1 ppm, and for workloads that consist of less than two hours a 0.20 ppm exposure is the limit, these are averaged over an 8-hour work shift (New Jersey Department of Health and Senior Services, 2003).

Chlorine Dioxide

Chlorine dioxide (ClO_2) is a yellow to reddish-yellow gas with a molecular mass of 67.45 g/mol and is a potent, selective oxidant that is applied as a disinfectant or sanitizer (Gordon & Rosenblatt, 2005; National Center for Biotechnology Information [NCBI], 2025). It is explosive in its pure gaseous or liquid form, although dilute aqueous solutions are stable (NCBI, 2025; Ortenberg & Telsch). Unlike chlorine, ClO_2 does not dissociate in water to form hypochlorous acid but remains as a dissolved gas in solution, maintaining its molecular structure and properties (Jefri et al., 2022). Exposure to light, particularly ultraviolet (UV) light, can decompose ClO_2 so minimizing light exposure is recommended (OxyChem, 2022). The antimicrobial activity in ClO_2 is primarily due to its strong oxidative action, which damages cell membranes, proteins, and nucleic acids. Because it is effective at low concentrations and produces fewer harmful by-products compared to chlorine (Jiang et al., 2024b), ClO_2 has become increasingly important in

disinfection and food industry applications. Similar to ozone, it is a powerful oxidizing agent, but its greater stability in aqueous systems makes it particularly advantageous for food processing and water treatment.

The use of ClO_2 has become more predominant over chlorine in pulp bleaching and its use in drinking water treatment has significantly increased (Gordon & Rosenblatt, 2005). There are important new applications for chlorine dioxide, particularly if it is applied as a gas (e.g., as an antimicrobial food packaging). The generation method is extremely important to the quality of the chlorine dioxide produced. A specific method of generation may be suitable for some applications, but not others. The choice of chlorine dioxide generation method, either aqueous or gaseous, should be decided based on the requirements of the microorganism and surface to ensure safety and effectiveness (Gordon & Rosenblatt, 2005).

Background

Chlorine dioxide (ClO_2) has been used for water disinfectant since 1904. It is widely used due to its high oxidation properties and efficiency in eradicating microorganism. Over time, chlorine dioxides use has expanded across various industries, particularly in the food industry, where sanitization and prevention of foodborne diseases are exceptionally important (Y. Zhang et al., 2023). In the meat industry, ClO_2 is added to the process water to reduce or inactivate pathogens that are found on the animal surface and intestinal tract (GO2 International, 2021). When applied in wash water, it removes bacteria, fungi, and other contaminants from fresh produce, seafood, and meats, thus reducing health risks for consumers and improving shelf life (GO2 International, 2021;

Whitmore, 2024a). Compared to chlorine-based disinfectant, ClO_2 can penetrate biofilms and kill all types of bacteria, viruses and fungi. Moreover, it does not produce trihalomethanes (THMs) and haloacetic acids (HAAs) as does chlorine, which are dangerous to health (Al-Otoum et al., 2016).

Chlorine dioxide is acknowledged for its ability to inactivate bacteria, viruses and controlling fungi in food processing. According to certain regulatory agencies, such as the U.S. Food and Drug Administration (FDA) as well as the Environmental Protection Agency (EPA), wherein they have set the limit of permissible residual chlorine dioxide concentration in drinking water and wash water. The EPA sets a maximum residual disinfectant level (MRDL) of 0.8 mg/L (ppm) for chlorine dioxide in drinking water (EPA, 2024), while the FDA permits its use for washing fresh produce at concentrations not exceeding 3 ppm residual chlorine dioxide (U.S. Food and Drug Administration [FDA], 2025). As an antimicrobial agent for washing produce, the FDA clears the use of chlorine compounds, but for application in water treatment and food processing plants, it is under the jurisdiction of the EPA, where regulations require residual levels not to exceed the EPA's drinking water standard of 0.8 ppm (Liestner, 2021; United States Department of Agriculture [USDA] & Agricultural Marketing Service, 2011). Likewise, the European Union (EU) has permitted the use of chlorine dioxide in food as sanitizers, but regulatory focus is placed on byproducts, with limits of 0.70 mg/L for chlorite and chlorate in water (European Parliament & Council of the European Union, 2020), and a default maximum residue level (MRL) of 0.01 mg/kg for

Table 3. Physical and Chemical Properties of Chlorine Dioxide.

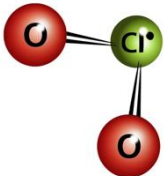
Property	Value	Reference
Molecular Formula	ClO_2	(National Center for Biotechnology Information, 2025)
Molecular Structure		(PureLine, 2024)
Color (gaseous)	Yellow-green and becomes redder at higher concentrations	(Agency for Toxic Substances and Disease Registry [ATSDR], 2004; PureLine, 2024)
Smell	Pungent, distinctive from chlorine	(ATSDR, 2004)
Aqueous Density	$\sim 1\text{g/mL}$	(PureLine, 2024)
Gaseous Density	2.4x heavier than air; and can accumulate in low-lying areas under ideal, still condition (controlled spaces)	(ERCO Worldwide, 2021; PureLine, 2024)

Table 3. Continued.

Molecular Weight	67.45 g/mol	(National Center for Biotechnology Information, 2025)
Vapor Pressure	96 KPa (720 mmHg) at 20°C (>1 atm)	(Occupational Safety and Health Administration [OSHA], 1990)
Oxidation Potential (V)	0.95 V	(ClorDiSys Solutions, 2014c)(Oxidation Technologies, 2024d)
Melting Point	-59°C (-74°F)	(National Institute for Occupational Safety and Health [NIOSH], 2019)
Boiling Point	11°C (52°F) at 760 mmHg	(NIOSH, 2019)
Heat of Combustion	-102.5 kJ/mol	(National Center for Biotechnology Information, 2025)
Solubility in Water	3.01 g/L at 25°C (0.3 % at 77°F and 34.5 mm Hg)	(NCBI, 2025; NIOSH, 2019)
Critical Temperature	-465°K	(NCBI, 2025)
Critical Pressure	8621.6 kPa	(NCBI, 2025)
Chemical Safety	 Oxidizer Compressed Gas Corrosive Acute Toxic Environmental Hazard	(National Center for Biotechnology Information, 2025)

chlorate in food (European Commission, 2020) ,ensuring microbial control without compromising food quality.

Method of Generation

Chemical Reaction Methods

There are many methods of producing chlorine dioxide, with one of the most common being the reaction of sodium chlorite (NaClO_2) with an acid. For instance, chlorine dioxide is readily generated when sodium chlorite reacts with acids such as hydrochloric acid (HCl), sodium bisulfate (NaHSO_4), citric acid ($\text{C}_6\text{H}_8\text{O}_7$), acetic acid (CH_3COOH), malic acid ($\text{HO}_2\text{CCHCH}_2\text{CO}_2\text{H}$), sulfuric acid (H_2SO_4) (Dong et al., 2022; H. Kim et al., 2008). This method is widely applied for ClO_2 synthesis because it is efficient and yields a high-purity product (Sales Monteiro et al., 2021). But, only about 65–68% actually ends up as ClO_2 gas while the remaining portion either lost to side reactions or left unreacted which minimizes byproducts such as chlorates (EAI Water, 2025a). This system is cost-effective for low-capacity applications, though they are less suitable for processes requiring high-output generation. The same principle underlies commercial powdered and two-part systems, where sodium chlorite is activated by an acid source to generate ClO_2 on site.

The one-step chlorine-chlorite method or the gaseous chlorine solids chlorite matrix, involves passing chlorine gas diluted with nitrogen (N_2) through a solid bed of sodium chlorite to produce ClO_2 gas, this process is capable of generating up to 1,200 lb of ClO_2 per day (EPA, 1999). Despite its efficiency, this method requires careful control

to prevent gas channeling through the solid bed, since it can result in the release chlorine gas during treatment (Science Applications International Corporation).

Another chemical method is the chlorine-chlorite method or also known as the two-step method. This method is flexible and efficient, it incorporates chlorine gas with sodium chlorite to obtain ClO_2 (EAI Water, 2025a; OxyChem, 2022). When chlorine is introduced into water it reacts to form hypochlorous acid (HOCl) and hydrochloric acid (HCl), these acids then react with sodium chlorite to yield chlorine dioxide, water, and sodium chloride but is necessary to use caution on using the reactant ratios since insufficient chlorine leaves residual chlorite, while excess chlorine promotes the undesired formation of sodium chlorate (OxyChem, 2022).

The three chemical method or the acid hypochlorite chlorite serves as an alternative to the chlorine–chlorite process in situations where chlorine gas is unavailable (OxyChem, 2022). It uses sodium chlorite, an acid, and sodium hypochlorite, the sodium hypochlorite is acidified to form hypochlorous acid, which subsequently reacts with the sodium chlorite to generate ClO_2 , the system is commonly employed by small to medium sized industrial facilities and food processors for water treatment (Whitmore, 2024b). The chlorine-chlorite method and the three chemical method achieve a high conversion efficiency of 95–98%, meaning that they are ideal for higher capacity applications, delivering a consistent production of chlorine dioxide (EAI Water, 2025).

Electrochemical Methods

Chlorine dioxide can be produced using electrochemical cells, where sodium chlorite (NaClO_2) is oxidized at the anode. This allows precise, on-demand generation of

ClO_2 , avoiding the need for transporting or storing large amounts of the gas. The reaction can happen directly at the anode or indirectly through hydroxyl radicals formed from water. One challenge is that dilute solutions can lead to overoxidation, producing unwanted chlorate ions (ClO_3^-), so designing electrodes that limit this side reaction is important (S. Zhao et al., 2025). Since electrochemical systems produce chlorine dioxide from a salt solution (sodium chloride) they can be used on demand, it eradicates the need for storing and handling hazardous chemicals on site (EAI Water, 2025b).

Electrochemical cells normally yields chlorine dioxide at 95–99% purity, with chlorite conversion exceeds 80%, this happens by relying on a single precursor, which is how it achieves high overall chemical efficiency and is economically favorable (EAI Water, 2025a). Electrochemical processes also introduce the concept of on-demand production to omit large scale storage of chlorine dioxide (Moratalla et al., 2022), though, one of the challenges of this method is that an overoxidation of chlorite to chlorate (ClO_3^-) can occur, especially when using dilute solutions, as the hydroxyl radicals can form at the anode and intensify the oxidation (S. Zhao et al., 2025). They are growing popular in fields where concentrations of working disinfectants are critical and their residual concentrations in water are important: the food industry, watering industry and other sectors when producing water.

Gaseous Generation

Chlorine dioxide may also be produced in gaseous form whereby chlorine gas (Cl_2) is passed through solid sodium chlorite solution. This method is quite useful when ClO_2 gas is needed, as in air purification and environmental disinfection in food

processing facilities (Y. Zhang et al., 2023) ClO_2 gas is extremely reactive thus it must be used as soon as it is produced to avoid its decomposition, this gas engages in oxidation reactions and quickly decomposes into chlorate (ClO_3^-) and chlorite (ClO_2^-) ions, which can subsequently be reduced to chloride (Cl^-) (Gómez-López et al., 2009). Not only that, but ClO_2 in gaseous form demonstrates stronger penetration capacity while producing lower levels of residual byproducts (EPA, 2007). The gaseous state of chlorine dioxide under certain concentrations also gives the potential of covering large areas and penetrating into the areas that are hard to reach, making it useful in preventing microbial contamination in stored or packaged foods.

Powder or Tablet

Chlorine dioxide (ClO_2) is also supplied as dry powders or tablets that generate ClO_2 . When the powder mixture comes into contact with water it releases ClO_2 on site rather than requiring bulk storage of the gas or a liquid concentrate. These products are used in many sectors, such as water treatment, food and beverage processing, agriculture, and bleaching applications, because of its ability to act as a disinfectant, sanitizer, and oxidizing agent (Pure Water, 2023). In the market, chlorine dioxide is available not only in powder form but also as tablets. Tablets operate through the same release mechanism as powders, typically incorporating sodium chlorite together with a solid acid, when they are submerged in water the components react to generate ClO_2 on demand. For instance, CD-TABSTM, from ClorDiSys Solutions, can produce a 100 ppm ClO_2 solution from a single tablet dissolved in one gallon of water, providing a convenient, non-acidic option for odor control and surface sanitization (ClorDiSys Solutions, 2014b). This approach

simplifies transport and can be easier to use without the cost required to purchase and install a generation system. Manufacturers (e.g., PureLine®, ICA Trinova, Safrax, SVS Aqua Technologies, ClorDiSys Solutions) offer powder and tablet-based systems for different scales and applications.

Chlorine dioxide powder offers several advantages over liquid formulations. It is lightweight and compact, making transport easier since no heavy equipment is needed, the powder form is generally more cost effective due to reduced production and shipping costs, and it allows for precise measurement and controlled dosing during treatment applications (Pure Water, 2023). While powder and tablet formulations are advantageous for portability and rapid use, they are less suited for situations that require a constant residual concentration.

Measurement and Units.

Spectrophotometry

The concentration of chlorine dioxide can be determined by spectrophotometry, either by measuring the absorbance of a water sample at around 360 nm, where dissolved ClO_2 absorbs better, or by measuring the absorbance in a gas-phase sample using a gas cell, or trapping the gas into a liquid (e.g., by bubbling air through water) and then measuring by spectrophotometry since gaseous ClO_2 also exhibits distinct absorbance features in that region (NCBI, 2004; Process Instruments (UK) Ltd., 2025). This technique depends on the knowledge that ClO_2 absorbs light in the UV and visible region usually about 360 nm (Sales Monteiro et al., 2021). According to Huang et al. (2018), differential absorbance at 316 nm and 400 nm (DA_{316} and DA_{400}) can be applied to

monitor changes in organic moieties during ClO_2 oxidation, in their study these spectral parameters correlated strongly with ClO_2 consumption and the formation of chlorite and chlorate. Ku (1933), stated in their research that ClO_2 shows higher absorbance in the visible UV range from about 500 to 275 nm, with an intensity maximum near 336 nm, plus infrared bands at 10.78, 10.44, 9.26–8.88, and 4.94 μm . Based to the intensity of the absorbed light, the concentration of chlorine dioxide in a particular volume of the sample can be measured with a high degree of accuracy.

Iodometric Titration

Iodometric titration is one of the traditional chemical titration methods that can be used to determine the concentration of chlorine dioxide by reacting with iodine. In this process ClO_2 oxidizes iodide (I^-) to iodine (I_2) which is latter reacted with sodium thiosulfate solution to establish the amount of chlorine dioxide present (Asakai, 2022). This method is suitable for determination of ClO_2 concentration in water and other liquid samples and it normally gives reliable results with little variance. Iodometric titration is another elaborate technique that can be relied upon; however, it involves high level of skill in handling the reagents and preparation of titrant in order to avoid interferences occasioned by other oxidants.

Electrochemical Sensors

Amperometric and potentiometric sensors are two electrochemical sensors that have been used for continuous monitoring of chlorine dioxide in liquid and gas phases. Amperometric sensors are devices which detect the current that results from the reduction or oxidation of ClO_2 at the electrode surface of the device, potentiometric sensors are

devices which measure the voltage depending on the concentration of ClO_2 (Janudin et al., 2022). Although these automation devices are normally used in the industrial areas as well as environmental monitoring because they offer a continuous measurement of the selected parameter, these sensors have a finite service life and exhibit calibration drift, so routine verification and periodic calibration/maintenance (per manufacturer guidance) are required. Sensors can drift and this can arise from membrane fouling, electrolyte depletion, electrode aging/poisoning, temperature/flow variability, and cross-sensitivities (e.g., free chlorine, ozone, chlorite) (Baracu & Dinu Gugoasa, 2021; Z. Zhang et al., 2023). Electrochemical sensors are very helpful in the food processing companies and water treatment plants because in such places routine disinfecting is carried out and accurate measurement of the disinfecting agent is very important.

Gas Detection Tubes

Gas detection tubes are portable devices used to help in measuring the concentration level of chlorine dioxide in the air in a short period of time. These tubes possess a chemical solution that reacts to ClO_2 and when it does there will be a chemical change (color change) dependent on the concentration level. The intensity of the change is indicative of concentration of the chlorine dioxide and can be used effectively to determine exposure of employees and the environment (Li et al., 2022). These tubes are widely used for spot checks of workplace air quality or to verify ClO_2 levels in food processing and storage areas, as well as treatment chambers. Although they are simpler and less expensive than laboratory-based techniques, gas detection tubes are semi-quantitative and less accurate, with reported precision typically around $\pm 20\%$ depending

on conditions and potential interfering gases (Honeywell International Inc, 2014).

Detection tubes are appropriate for rapid screening assessments, while laboratory methods are preferred for compliance monitoring or detailed exposure studies.

To compare the results reported in literature, is often necessary to convert the concentrations expressed in mg/L (mass per volume of solution) into ppmv (parts per million by volume). The relationship is based on the ideal gas law: $PV = nRT$. Where P is pressure, V is volume, n is the number of mol, R is the universal gas constant, and T is temperature expressed in kelvins (°K). The number of mols is first determined using mass concentration: $n = \frac{\text{mass in mg}}{1000 * M_{\text{ClO}_2}}$, where M_{ClO_2} is 67.45 g/mol. Substituting into the ideal gas equation at 25°C or 298.15°K at 1 atm: $PV = nRT =$

$$\frac{\left(\frac{\text{mass in mg/L}}{1000 * 67.45 \text{ g/mol}} \right) * 0.08206 \frac{\text{L*atm}}{\text{mol*K}} * 298.15 \text{ K}}{1 \text{ atm}} = \frac{\text{mass in mg/L} * 0.08206 \frac{\text{L*atm}}{\text{mol*K}} * 298.15 \text{ K}}{1000 * 67.45 \text{ g/mol}}$$

This equation provides the gas concentration in ppmv relative to the total volume of air and according to Committee on Acute Exposure Guideline Levels et al. (2007), 1 ppmv of $\text{ClO}_2 = 2.76 \text{ mg/m}^3$ and 1 mg/L of $\text{ClO}_2 = 362 \text{ ppmv}$.

Chlorine Dioxide: Gaseous vs. Aqueous

Chlorine dioxide (ClO_2) can be applied either as a gas in air or dissolved in water (aqueous ClO_2), with each form having distinct advantages depending on the intended application. According to the OxyChem (2022) Health & Safety datasheet, gaseous ClO_2 is stable in air at concentrations below 10% by volume, but it becomes highly unstable and explosive at higher levels, particularly when it is exposed to heat, light, or oxidizable materials. In contrast, aqueous ClO_2 solutions are relatively more stable when they are

properly stored, ideally in acidic conditions (below pH levels of seven), kept cool, and protected from UV light.

Gaseous Chlorine Dioxide

Gaseous ClO₂ is highly effective for disinfecting surfaces and the air in enclosed areas because it can reach locations where liquid disinfectants cannot penetrate due to surface tension effects (Sun et al., 2019) and is widely applied across diverse industries due to its strong disinfecting and oxidizing properties. In the healthcare, ClO₂ gas is used for the sanitation of medical equipment, rooms, and surfaces. In the food and beverage sector, it is applied to production lines, storage areas, packaging facilities, and air spaces (Whitmore, 2024b)

Gaseous ClO₂ has been used successfully in the dairy, produce, meat, grain, spice, frozen food, and beverage industries. It provides residue-free decontamination of facilities, tanks, laboratories, spiral freezers, piping systems, containers, and even cargo trailers (ClorDiSys Solutions, 2014a; Lenntech, 2025). As a result, it is considered a safe and versatile method for eliminating microorganisms on food-contact surfaces and even entire processing facilities in the gaseous state.

Aqueous Chlorine Dioxide

In contrast, aqueous ClO₂ is primarily used for direct food contact applications. It is incorporated into rinses, dips, and sprays for washing fruits, vegetables, fish, and meats, as well as for sanitizing food-contact equipment (Y. Zhang et al., 2023). Industrial uses include sewage water disinfection, process water treatment, cooling towers, industrial air treatment, and mussel control. It is also applied in animal housing

sanitation, pulp and paper bleaching, and the oil and gas industry for water purification (Parks, 2023; Scotmas, 2023)

Aqueous solutions provide effective microbial reduction while staying within regulatory safety thresholds. Compared with traditional chlorine-based disinfectants, aqueous ClO_2 is preferred in the food industry because it does not produce harmful chlorinated byproducts (Ren et al., 2023). It is also more affordable and easier to apply in routine sanitization processes such as packaging, food storage, and processors where aerosolization may pose a concern (Singh et al., 2021).

Factors Influencing Chlorine Dioxide Efficiency

Factors such as gas concentration, relative humidity, treatment time, and temperature can affect the disinfection rate of gaseous ClO_2 . Relative humidity (RH) plays a critical role in the efficacy of gaseous ClO_2 , since a high RH allows ClO_2 to dissolve and penetrate microbial membranes or biofilms using low concentration. This does not mean that it is not effective at low RH levels (45%, 55%, and 65%), just that higher dosage of ClO_2 is required (Lorcheim, 2021). Studies show that maintaining RH levels between 75% and 90% significantly improves the efficiency of gaseous ClO_2 at low concentrations, often reducing microbial populations to below detectable limits (S. Kim & Park, 2022; Shirasaki et al., 2016; Yi et al., 2016). ClO_2 gas remains effective in moist environments, so surfaces do not need to be completely dry after cleaning or wash downs for decontamination to work. Since the gas disperses evenly, it can reach all areas of a space regardless of size, height, or equipment present (Lorcheim, 2021).

Contact time affects the performance of ClO_2 as a disinfectant, longer exposure allows ClO_2 to penetrate biofilms or surface crevices, enhancing the destruction of bacteria, spores, and fungi (Jefri et al., 2022). Factors like temperature and pressure influence the physical stability of ClO_2 . While elevated temperatures can enhance reaction kinetics, ClO_2 becomes thermally unstable above approximately 50°C and tends to decompose, reducing its efficacy (Jiang et al., 2024a). In contrast, low temperatures improve gas stability but may slow disinfection kinetics and risk condensation. ClO_2 has a boiling point of about 11°C (52°F) at 1 atm, meaning below this threshold it may liquefy (ERCO Worldwide, 2021; OxyChem, 2022). Although pressure does not directly impact antimicrobial activity, it plays a critical role in determining the physical state of ClO_2 (e.g., gaseous or aqueous), thus influencing its storage, transport, and safe handling.

Other environmental factors, such as the pH of the water being treated, the organic load, and the type of contact surface, also affect the efficacy of ClO_2 . Because of its high solubility in water, ClO_2 does not hydrolyze into ions and because of this it retains its oxidizing and antimicrobial properties over a broad pH range from four to ten (International Dioxide, 2025). However, in alkaline conditions (pH levels above eight), ClO_2 solutions decompose, undergoing hydrolysis that produces chlorate and chlorite ions. , In contrast, acidified solutions (pH levels below seven) can remain stable for several months when stored in a cool, well-ventilated environment, protected from light, heat, or ignition sources (OxyChem, 2022). The presence of organic matter, such as food residues or proteins, can reduce the effectiveness of ClO_2 , because the gas reacts not only with microbial cells but also with surrounding organic material. This competition

decreases the amount of ClO₂ available for microbial inactivation, often requiring higher doses to achieve the desired disinfection outcome (Hupperich et al., 2020; Swietlik et al., 2004). Microbial inactivation is also influenced by the physical characteristics of the contact surface. Smooth, non-porous surfaces are easier to sanitize, while rough or porous surfaces can shelter microorganisms in crevices, limiting exposure to ClO₂ (Park & Kang, 2017).

Risks of Exposure and Safety Limits

Chlorine dioxide (ClO₂) applied as a gas in air is a potent disinfectant but must be carefully applied due to hazards resulting from inhalation. The Occupational Safety and Health Administration (OSHA) Permissible Exposure Limit (PEL) for ClO₂ is 0.1 ppm over an 8 hour period as a time-weighted average (TWA) which ensures that exposure to the compound for a long time does not rise to dangerous levels (Liestner, 2021). The National Institute for Occupational Safety and Health (NIOSH) states that exposure limit for ClO₂ is 0.1 ppm over a 10 hour shift with a short-term exposure cap of 0.3 ppm for any 15 minute period while the American Conference of Governmental Industrial Hygienists (ACGIH) also recommends that the exposure limit for ClO₂ should be 0.1 ppm but over a 8 hour shift and a short term exposure limit (STEL) of 0.3 ppm (New Jersey Department of Health and Senior Services, 2003). The short-term exposure limit (STEL) of 0.3 ppm for a 15-minute time-weighted average is to avoid health impact of higher concentration exposure, such limits assist in eliminating some of the hazards of employing ClO₂ as a gas in confined spaces (Hassel et al., 2022).

Chlorine dioxide is toxic and causes adverse health complications particularly on the respiratory system when one is exposed to it for long times or in large amounts. High concentrations of ClO₂ gas can affect the eyes, throat and lungs, provoking irritation, cough, difficult breathing and in extreme cases pulmonary oedema (Andrés et al., 2022). Personnel working around ClO₂ should have safety guidelines such as provisions for proper ventilation, personal gas detectors and respirators and gloves among others. In the food processing industry, some of the ways of managing exposure risks are through high automation of dosing, restricting access to sealed off treatment areas, and adequate ventilation of the ClO₂ gas after treatment.

Comparison of Chlorine Dioxide and Ozone for Log Reduction of *Escherichia coli*

Ozone (O₃) and chlorine dioxide (ClO₂) are both recognized as highly effective unstable, oxidizing agents for the inactivation of *Escherichia coli*, yet they differ in their mechanisms of action and residual activity. Chlorine dioxide shows high inactivation effectiveness at relatively low concentrations by selectively oxidizing cellular proteins and disrupting membrane integrity (Tao et al., 2024). An advantage of ClO₂ is its ability to maintain residual activity in water, allowing extended protection against microbial regrowth (Chai et al., 2022). In contrast, ozone causes rapid, non-specific damage to the bacterial cell walls, and it decomposes within minutes and leaves no residue to continue to act (Hossen et al., 2023). While this limits a long-term effectiveness, ozone's high reactivity can achieve rapid log reductions in microbial populations and has gained increasing interest for improving safety and shelf life of food (Guzel-Seydim et al., 2004; National Research Council (US) Safe Drinking Water Committee, 1980). ClO₂ is favored

when constant antimicrobial protection is required, whereas ozone is more suitable for rapid, on-demand disinfection.

Chlorine dioxide's mechanism of inactivation works by damaging the cell membrane of the bacteria and stopping important cell functions (e.g., the production of proteins) (Malka & Park, 2021). It reacts with oxygenated compounds and proteins found in the cell membrane, this alters the cell metabolism, basically breaking the cells balance and energy flow (Praeger et al., 2018). Chlorine dioxide oxidizes certain groups on the cell surface proteins, called the sulfhydryl groups, which in return weaken the membrane and make it leak (Malka & Park, 2021). This allows things like potassium ions (K^+) to leak out, thus destroying the cells' ability to control what goes in and out (permeability control), this is one of the key reasons why *Escherichia coli* cells die (Berg et al., 1986). Even though chlorine dioxide causes these internal problems, studies of electron microscope show that there is no significant morphological damage or cell lysis (cells may not look broken or burst) (Bridges et al., 2020; Malka & Park, 2021; Ofori et al., 2018). Furthermore, chlorine dioxide has a stronger reaction with amino acids, the order of most to least reactive is cysteine, tyrosine, tryptophan, histidine, and proline (V. K. Sharma & Sohn, 2012).

On the other hand, ozone's mechanism of inactivation is done through lysis, in where the breakdown of the cell occurs due to the rupture of its wall or membrane causing the loss of structural integrity. During the breakdown of ozone (O_3) to oxygen (O_2), ozone generates a strong oxidative burst that it damages any bacterial cell that is nearby. This oxidation attacks all vital cellular components (e.g., membrane lipids,

proteins, enzymes, and nucleic acids) resulting in the disruption of the membrane that causes the cell to leak out its contents, leading to death (Oxidation Technologies, 2024b). Although they may be remaining dead bacteria cells, ozone inactivates all viable bacteria. Because its antimicrobial action depends on oxidation, ozone is effective against a broad range of microorganisms, and no bacteria are known to have developed resistance to it (Oxidation Technologies, 2024b).

Ozone Gas and Chlorine Dioxide Gas Compared at Equivalent Oxidizing

Concentrations

Ozone gas and chlorine dioxide have been compared for their antimicrobial effectiveness on various surfaces. Most comparative studies rely on CT values (concentration $\times$ time) or use concentrations chosen independently for each gas. Consequently, there is limited information about evaluating their antimicrobial efficacy under equivalent oxidative capacities, an approach that may allow a more accurate comparison of their log reduction performance.

Ozone and chlorine dioxide are both strong oxidizing agents, but they differ in redox strength, reaction selectivity, and sensitivity to environmental conditions. Ozone has a higher oxidation potential (2.07 V) and acts as a rapid, non-selective oxidant, whereas chlorine dioxide possesses a lower oxidation potential (0.95 V) but reacts in a more selective manner (United Nations Environment Programme et al., 2000). The oxidation capacity was chosen to determine the equivalence concentration of gaseous ozone and chlorine dioxide, because this takes into account the difference in redox strength between ozone and chlorine dioxide.

Trinetta et al. (2011) compared ozone and chlorine dioxide gas treatments against *Escherichia coli* O157:H7 and *Salmonella enterica* that were inoculated onto tomato, cantaloupe, and lettuce seeds. Both gases significantly reduced microbial loads; however, under specific relative humidity and exposure conditions, ozone outperformed chlorine dioxide at similar applied concentrations (ozone obtained a log reduction of 3.53 and chlorine dioxide obtained a log reduction of 1.94). While in the study of Bridges et al. (2018), they evaluated closed-circulation gaseous treatments of *Escherichia coli* O157:H7, *Salmonella* serovars, and *Listeria monocytogenes* inoculated onto carrots, blueberries, or tomatoes. They observed that ClO₂ achieved a much larger log reduction (> 7 log) than O₃ (< 2 log) under a scaled-up closed-circulation treatment system, ClO₂ demonstrated to more efficient than ozone.

CHAPTER THREE

MATERIALS AND METHODS

Bacterial Culture

The bacterial strain used in this investigation was *Escherichia coli* strain ATCC 11229 AMC 198. The strain was stored at -80°C in 2 mL vial tubes containing Luria-Bertani (LB) broth supplemented with 15% glycerol. A frozen stock was thawed to room temperature and poured into 100 mL of LB broth, then incubated at 37°C for 24 hours in static conditions. Following this, 10 µL of the culture was transferred into 10 mL of fresh LB broth and incubated at 37°C for 24 hours under static conditions. After a third transfer and 24 h incubation, the culture was centrifuged at 7,000g for 20 minutes, the supernatant was removed, and the cell pellet was resuspended in 10 mL of physiological saline to prepare the cell suspension. The optical density at 600 nm (OD₆₀₀) was measured using a spectrophotometer to estimate cell concentration prior to each use. The culture was used after daily transfers for up to two weeks and at the end, the process was restarted with a new frozen stock.

Coupons Preparation

Stainless steel coupons (ASTM 316) measuring approximately 3 cm × 2 cm, were cleaned by soaking in phosphate-free soap for 15 minutes, followed by scrubbing with a non-abrasive brush, and thoroughly rinsed with MILLIQ deionized water. They were then soaked in 5% hydrochloric acid (HCl) for 20 minutes to remove surface contaminants, rinsed again with MILLIQ deionized water, and air-dried overnight in a biosafety cabinet, this method was adapted from Arnold and Mitchell (2002), Chen and

Snyder (2023), and L. Zhao et al. (2017). During drying, coupons were exposed to ultraviolet (UV) light (254 nm) for 20 minutes to sanitize the surface, based on the paper of Katara et al. (2008). The sanitization procedure for coupons was verified by incubation for 24 hours after covering with nutrient agar.

Inoculation of Bacterial Culture onto Stainless Steel Coupons

The coupons were inoculated using the cell suspension from the overnight culture of *E. coli* that had an approximate initial concentration of 10^9 CFU/mL, as determined by serial dilution and plate counting. A 100 μ L aliquot of the cell suspension was divided into 10 droplets and applied onto the surface (6 cm² area) of each stainless-steel coupon using a sterile pipette tip to ensure even distribution. Inoculated coupons were placed in Petri dishes for drying.

Drying Process

Inoculated coupons were placed in a plastic box containing a controlled humidity environment. A saturated potassium carbonate solution below a plastic mesh was used to maintain a relative humidity (RH) range of approximately 42.5% to 56% within the box. Air circulation was managed by a small fan to ensure uniform conditions, and a magnetic stirrer was used to agitate the saturated solution below the coupons. The drying process was conducted at room temperature ($19.9 \pm 1.6^\circ\text{C}$) for 2 hours. Upon drying, the coupons were removed and randomly designated as either control or treatment coupons.

Humidity Treatment

All dried coupons were subjected to pre- and post-humidification for 10 minutes and 20 minutes, respectively, in a plastic box designed to maintain a controlled humidity

environment. This step was included to equilibrate the samples to a consistent moisture level, as surface hydration can strongly influence gas penetration, microbial susceptibility and recoverability, as previously reported by Record et al. (1962) and Louis et al. (1994). A container filled with distilled water sustained a relative humidity between approximately 89% to 92%. A small fan facilitated air circulation to ensure uniformity, and a magnetic stirrer agitated the MILLIQ deionized water to stabilize RH levels.

Ozone Gas and Chlorine Dioxide Gas Generation and Coupon Treatment

Stainless steel coupons were placed in a sealed chamber (large glove box) for treatment. For ozone treatment, ozone was generated via corona discharge (dielectric barrier discharge (DBD) generators) using an oxygen gas tank (99% purity) and pumped into the chamber. The chamber had a single opening, covered by plastic using magnets to hold it in place, the plastic was lifted only to transfer coupons in and out, ensuring minimal gas leakage. Two internal fans provided air circulation during treatment of coupons. A carbon scrubber (blower attached to a drum of activated carbon) was used to remove residual gases from the chamber after each treatment. For chlorine dioxide treatment, gas was generated by passing a compressed gas (2% chlorine in nitrogen) through a PVC cartridge filled with technical sodium chlorite (~ 80% sodium chlorite) with tubing leading into the chamber. All other procedures, including circulation and gas scrubbing, were the same as for ozone gas treatment.

A humidifier (brand, Vicks 1.5 gal 150 sq ft Manual Ultrasonic Cool Mist Humidifier) regulated relative humidity levels within the chamber, at a normal atmospheric pressure, and no significant variation from room temperature ($19.9 \pm 1.6^{\circ}\text{C}$).

In each test condition, two coupons for control coupons (exposed to humidity only) and two coupons treatment (exposed to ozone gas and humidity) were included. Ozone gas was tested at concentrations of 15, 50, 75, 100, and 125 ppmv for 30 minutes at RH levels of approximately $66.7\% \pm 0.6\%$, $75.3\% \pm 1.1\%$, and $91.1\% \pm 1.3\%$. Ozone concentration was monitored during treatment using a PortaSens III portable gas leak detector (Analytical Technology, Inc. Collegeville, PA) and verified during each treatment using a No. 18EL Gastec detection tube or a Dräger Tube Ozone 10/A. Ozone was manually added by momentarily opening the valve on the oxygen gas cylinder to increase ozone levels in the chamber when needed. Control coupons were placed in a plastic box with a container with distilled water to replicate humidity conditions without gas exposure. Once treatment was completed, coupons were subjected to bacteria recovery and enumeration procedures.

Bacteria Recovery, Enumeration, and Calculation of Estimated D-value

Treated and control coupons were individually transferred to 50 mL centrifuge tubes, each containing 32 mL of sterile physiological saline (PS) (w/v) with 1% Tween 80 (v/v). Sodium thiosulfate was added to the recovery solution after chlorine dioxide treatment. Coupons were left soaking for 30 minutes and vortexed vigorously for 1 minute to facilitate the recovery of the bacteria from the coupon surface. The solution was serially diluted in 9 mL PS tubes, and 100 μ L of the dilutions were spread-plated in duplicate onto Nutrient Agar (NA). Plates were incubated at 37°C for 24 hours, and colony-forming units (CFU) were counted to assess the survival after treatment.

The reduction of the *E. coli* cells inoculated onto stainless steel was expressed as log reduction, showing the decrease of cells after treatment compared to the untreated coupons. Log reduction was calculated by using the following formula: $\log reduction = \log_{10}\left(\frac{N_0}{N_t}\right)$. Where, N_0 is the average CFU/coupon recovered from the control coupons and N_t is the average CFU/coupon recovered from the treatment coupons.

The estimated decimal reduction time (estimated D-value) was obtained by using $D_{estimated} = \frac{t}{N}$. This estimated D-value represents an approximation of the time required to achieve a 1 log₁₀ (90%) reduction in *E. coli* cells under a treatment period of 30 minutes.

Determination of Chlorine Dioxide Equivalent Concentration to Ozone

The equivalent concentration of chlorine dioxide was calculated from the applied ozone concentration using a 2:5 ratio (2 moles O₃: 5 moles ClO₂). Using this ratio, the equivalent concentration of chlorine dioxide was calculated from the applied ozone concentration according to the following expression: $ClO_{2ppmv} = O_{3ppmv} * \frac{2O_3}{5ClO_2}$. This ratio was selected to provide a comparable oxidative exposure between treatments, allowing both gases to be evaluated under similar treatment conditions.

Statistical analysis

Experiments were performed in triplicate. Log₁₀ reductions of *E. coli* counts were calculated by comparing the surviving population after treatment with the population recovered on control coupons. Statistical analyses were performed using JMP (SAS Institute INC., Cary, NC, USA). The data was tested for normality of variance using the Shapiro-Wilk test. To evaluate the effect of relative humidity on microbial inactivation

within each concentration a one-way analysis of variance (ANOVA) was used. Then, using Tukey's Honestly Significant Difference (HSD) post hoc test, the log reduction means were compared across humidity levels within each concentration. While a two-way analysis of variance (ANOVA) was used to evaluate the effects of ozone concentration and relative humidity (RH %) on the inactivation of *Escherichia coli*. When significant effects were detected ($p \leq 0.05$), a Tukey's HSD post hoc test was performed for mean separation. To compare the log reduction of ozone and chlorine dioxide at equivalent concentrations, the data was analyzed using a mixed model analysis. Where gas type, concentration, and relative humidity were treated as fixed effects, while experiment ID was included as a random effect to account for experimental variability. Significance was assessed at $p \leq 0.05$, and results are expressed as mean $\pm$ standard deviation (SD).

CHAPTER FOUR

RESULTS AND DISCUSSION

In this chapter the results from gaseous ozone (O₃) and gaseous chlorine dioxide (ClO₂) treatments on *Escherichia coli* inoculated onto stainless steel coupons are presented. The results are divided into three sections: the effect of ozone concentration on the inactivation of *Escherichia coli* under high relative humidity (RH 91.1%), the influence of humidity and ozone concentration on the inactivation of *Escherichia coli*, and the comparison of ozone and chlorine dioxide applied at equivalent oxidative concentrations. All gas treatments were applied for 30 minutes to duplicate coupons and performed in triplicate.

Results

Effect of ozone concentration on the inactivation of Escherichia coli under high relative humidity (91.1%)

In **Figure 1**, increasing ozone concentration resulted in higher *E. coli* reductions when treatments were performed at high relative humidity ($91.1 \pm 1.3\%$) for 30 minutes. At 15 ppmv, the overall reduction was low, with an average of 0.6 ± 0.3 log. Increasing the concentration to 50 ppmv improved the reduction to 1.6 ± 0.3 log. Ozone achieved an inactivation of 2.0 ± 0.3 log and 2.7 ± 0.8 log at 75 and 100 ppmv, respectively. The highest concentration of ozone at 125 ppmv ($91.1 \pm 1.3\%$ relative humidity), resulted in 3.6 ± 0.9 log reduction of *E. coli*. The results corresponding to **Figure 1** are summarized in **Table 4**, which presents the mean log reduction of *Escherichia coli* after 30 minutes ozone treatment.

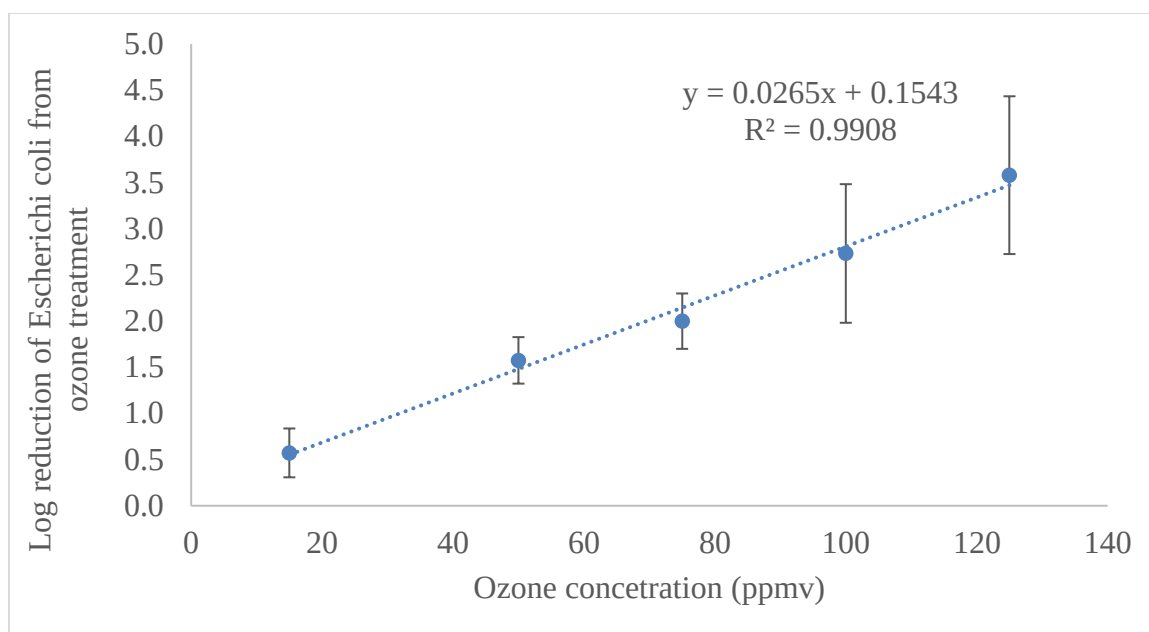


Figure 1. Log reduction of *Escherichia coli* on stainless-steel coupons after treatment with ozone gas (15–125 ppmv) for 30 minutes under high RH ($91.1 \pm 1.3\%$). Error bars represent mean $\pm$ SD ($n = 3$). A linear relationship was observed and described by $y = 0.0265x + 0.1543$ ($R^2 = 0.9908$).

The ANOVA showed that the ozone concentration had a significant effect on microbial inactivation ($p = 0.0028$), while relative humidity was not significant ($p = 0.7954$). These findings agree with previous work showing that ozone inactivation on solid surfaces are higher depending on concentration and contact time (Wood et al., 2020). However, the lack of significance of relative humidity may be due to the sample pre-humidification procedures applied and/or the high variation across replicate experiments.

Figure 2 compares the recoverable *E. coli* populations from treated coupons and the untreated control coupons. Untreated controls consistently maintained a recovery of approximately 10^6 - 10^7 CFU/coupon, this confirms that *E. coli* cells survived the drying

Table 4. Effect of Ozone Concentration on the Log Reduction of *Escherichia coli* on Stainless Steel Coupons under High Relative Humidity ($91.1 \pm 1.3\%$).

Ozone concentration (ppmv)	Log reduction after ozone treatment (CFU/coupon) (mean $\pm$ SD)
15	0.6 ± 0.3^C
50	1.6 ± 0.3^{BC}
75	2.0 ± 0.3^{ABC}
100	2.7 ± 0.8^{AB}
125	3.6 ± 0.9^A

Values are presented as mean $\pm$ standard deviation (n = 3). Different letters indicate significant differences between log reduction at $p < 0.05$ at different ozone concentrations.

and humidity conditions. Drying alone resulted in an approximate log reduction of 1.5 log, which is based on the difference between the initial inoculum concentration and the counts recovered from the untreated control samples. While treated coupons showed a recovery of approximately 10^3 - 10^6 CFU/coupon, showing that the ozone treatment reduced the population. The difference between the control coupons and the treated coupons reflects solely the effect caused by ozone.

Ozone concentration influenced the inactivation of *Escherichia coli* for all humidities tested. The following section examines the effect of relative humidity on log reduction within each ozone concentration.

Influence of Relative Humidity on the Log Reduction of Escherichia coli Using Gaseous Ozone

As shown in **Table 5**, **Table 6**, **Figure 3**, and **Figure 4**, ozone concentration and relative humidity influenced the inactivation of *E. coli*. ANOVA results confirmed that relative humidity and ozone concentration were statistically significant on microbial

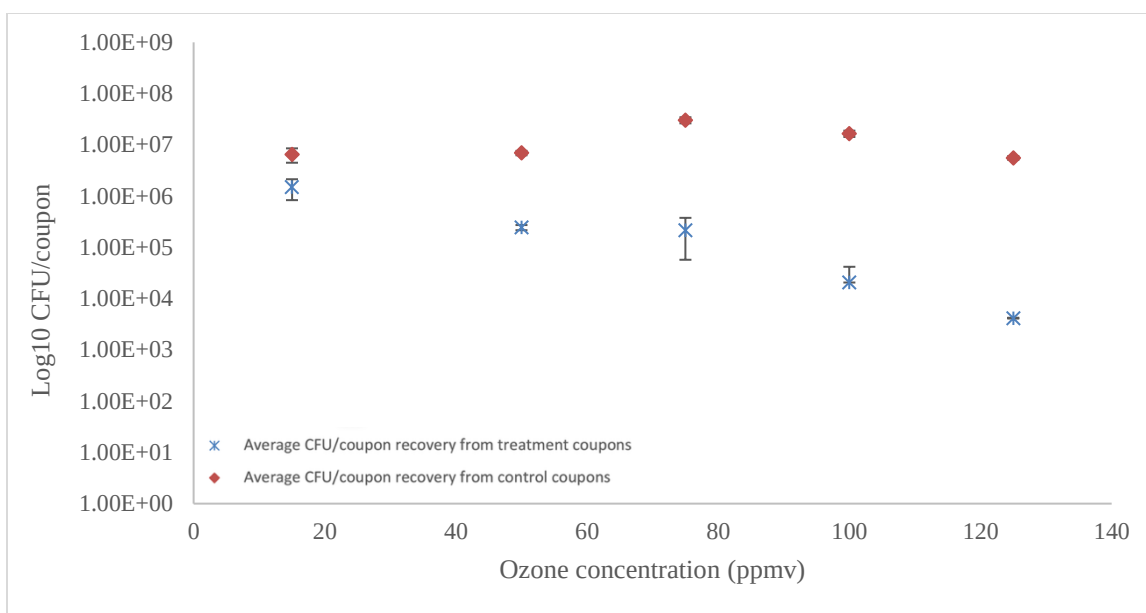


Figure 2. Comparison of recovered *E. coli* (CFU/coupon) from treated and untreated stainless-steel coupons across ozone gas concentrations (15–125 ppmv) for 30 minutes under high relative (91.1 ± 1.3%). Error bars represent mean ± SD (n = 3).

inactivation ($p < 0.0001$ and $p < 0.0289$, respectively). But the interaction between ozone concentration and relative humidity was not significant ($p = 0.2977$), indicating that both factors acted independently. Although the interaction between relative humidity and ozone concentration was not statistically significant, higher humidity levels still resulted in greater inactivation at all ozone concentrations tested.

At low relative humidity ($66.8 \pm 0.6\%$), the inactivation was the lowest which ranged from 0.9 to 1.2 log across 75 ppmv, 100 ppmv, and 125 ppmv. When relative humidity increased to $75.3 \pm 1.1\%$, log reductions improved to 1.5-1.9 log across 75 ppmv, 100 ppmv, and 125 ppmv. But the greatest inactivation occurred at a relative humidity of $91.1 \pm 1.3\%$, where 75, 100, and 125 ppmv ozone achieved mean reductions of 2.0 ± 0.3 , 2.7 ± 0.8 , and 3.6 ± 0.9 log, respectively (**Table 5** and **Table 6**). These three

concentrations were selected for comparison because at lower ozone levels, such as 15 ppmv and 50 ppmv, a noticeable log reduction might not have been observable under low relative humidity.

Table 5, show the results of the effect relative humidity had on the inactivation of *Escherichia coli* within each concentration. It can be seen in **Table 5** that at 75 and 100 ppmv, the log reductions at 75.3% relative humidity were not statistically significantly different from those obtained at 66.8% or 91.1%. This indicates that, at 75 and 100 ppmv, the use of a relative humidity of 75.3% was as effective in inactivating *E. coli* as using low and high relative humidity. However, at 125 ppmv, the log reductions at 75.3% relative humidity were statistically similar to the ones achieved at 66.8%, but at 91.1% relative humidity log reductions were higher.

Table 5. Effect of relative humidity on the log reduction of *E. coli* within each ozone concentration (75, 100, and 125 ppmv O₃).

RH Level	75 ppmv O ₃	100 ppmv O ₃	125 ppmv O ₃
	Mean Log Reduction $\pm$ SD		
66.8 $\pm$ 0.6% (Low RH)	0.9 $\pm$ 0.2 ^B	1.1 $\pm$ 0.5 ^B	1.2 $\pm$ 0.2 ^B
75.3 $\pm$ 1.1% (Medium RH)	1.5 $\pm$ 0.7 ^{AB}	1.7 $\pm$ 0.3 ^{AB}	1.9 $\pm$ 0.7 ^B
91.1 $\pm$ 1.3% (High RH)	2.0 $\pm$ 0.3 ^A	2.7 $\pm$ 0.8 ^A	3.6 $\pm$ 0.9 ^A

Values are presented as mean $\pm$ standard deviation (n = 3). Different letters indicate significant differences at log reduction at p < 0.05.

While **Table 6** compares all combinations of individual treatments. Here it is presented how each specific combination of ozone concentration and humidity varied from each other in terms of log reduction. At both 66.8% and 75.3% relative humidity,

ozone concentrations of 75, 100, and 125 ppmv were not statistically significantly from each other, which can be confirmed through by the shared letter grouping of CD (66.8%) and BCD (75.3%). This suggests that, at low and medium humidity, the increase in concentration did not produce significant levels of inactivation.

At 91.1% RH, ozone concentrations of 100 ppmv did not differ from 75 and 125, although both 100 and 125 ppmv achieved higher log reductions than 75 ppmv, increasing 0.7 with 100 ppmv and 1.6 with 125 ppmv. It should be pointed out that using 50 ppmv of ozone at 91.1% RH (1.6 log) can achieve similar log reductions as 75, 100, and 125 ppmv under 75.3% RH, which can reflect the strong impact that humidity posed during treatment. Across all treatments, 15 ppmv ozone at 91.1% RH achieved the lowest log reduction (0.6 log), representing the least effective treatment condition tested.

Table 6. Log reduction of *Escherichia coli* on stainless steel coupons treated with ozone gas (15, 50, 75, 100, and 125 ppmv) under varying relative humidity levels ($66.8 \pm 0.6\%$, $75.3 \pm 1.1\%$, and $91.1 \pm 1.3\%$).

RH Level	15 ppmv	50 ppmv	75 ppmv	100 ppmv	125 ppmv
$66.8 \pm 0.6\%$ (Low RH)	N/A	N/A	0.9 ± 0.2^{CD}	1.1 ± 0.5^{CD}	1.2 ± 0.2^{CD}
$75.3 \pm 1.1\%$ (Medium RH)	N/A	N/A	1.5 ± 0.7^{BCD}	1.7 ± 0.3^{BCD}	1.9 ± 0.7^{BCD}
$91.1 \pm 1.3\%$ (High RH)	0.6 ± 0.3^D	1.6 ± 0.3^{BCD}	2.0 ± 0.3^{BC}	2.7 ± 0.8^{AB}	3.6 ± 0.9^A

Values are presented as mean $\pm$ standard deviation (n = 3). Different letters indicate significant differences at log reduction at $p < 0.05$. N/A = not available.

To obtain a better visualization of the results, **Figures 3** and **Figure 4** were generated. Both represent the same data that was presented in **Tables 5** and **Table 6** but, the graphs grouped the reductions by relative humidity (**Figure 3**) or by ozone concentration (**Figure 4**).

Figure 3 illustrates that in all ozone concentrations, log reduction increased as relative humidity increased. Values were grouped by relative humidity levels, where each line shows progressively higher reductions from low to high RH to show how ozone concentration affected the inactivation of *E. coli* cells.

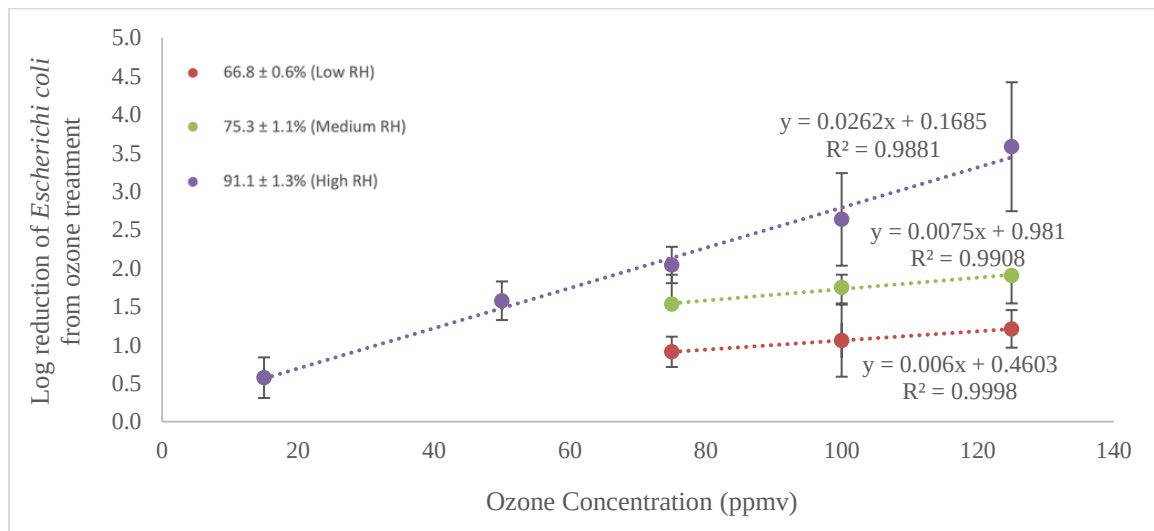


Figure 3. Log reduction of *Escherichia coli* on stainless-steel coupons treated with ozone gas (15, 50, 75, 100, and 125 ppmv) under three relative humidity conditions ($66.8 \pm 0.6\%$, $75.3 \pm 1.1\%$, and $91.1 \pm 1.3\%$). Each line represents a different relative humidity.

In **Figure 4**, values were grouped by ozone concentration, where each line shows progressively higher reductions from low to high RH to show how relative humidity affected the microbial inactivation. The largest separation between humidity levels occurs at 100 and 125 ppmv, demonstrating that RH has a higher impact at higher ozone doses.

Tukey's multiple comparison test confirmed that the treatments performed at 91.1% relative humidity resulted in a higher inactivation compared to the treatments at relative humidities of 75.3% and 66.8%. On average, ozone obtained a log reduction of 2.7 log at 91.1% RH, 1.73 log at 75.3% RH, and only a 1.06 log at 66.8% RH for all concentrations used. This shows that humidity has a stronger effect when the concentrations of ozone are higher.

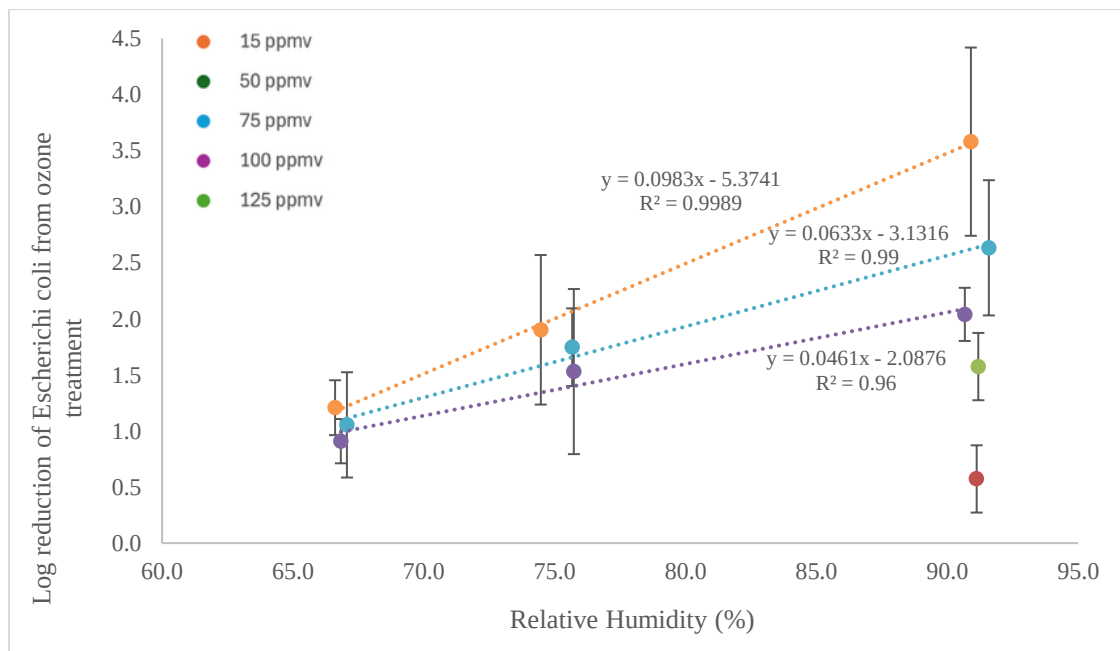


Figure 4. Influence of relative humidity on inactivation of *Escherichia coli* at different ozone concentrations. Log reduction values are shown at $66.8 \pm 0.6\%$, $75.3 \pm 1.1\%$, and $91.1 \pm 1.3\%$ RH for 15, 50, 75, 100, and 125 ppmv ozone exposure. Each line represents a specific ozone concentration, and error bars represent mean $\pm$ SD ($n = 3$).

Comparison of Ozone and Chlorine Dioxide at Equivalent Oxidative Concentrations

Table 7 summarizes the log reduction of *E. coli* achieved by using ozone and chlorine dioxide at equivalent oxidative concentrations under low ($66.8 \pm 0.6\%$) and high

(91.1 ± 1.3%) relative humidity conditions. The equivalent doses were calculated using the equation $ClO_{2ppmv} = O_{3ppmv} * \frac{2O_3}{5ClO_2}$, which is based on the oxidative capacity ratio of 2 moles of O₃ to 5 moles of ClO₂. Using this ratio, the equivalent concentrations are 75 ppmv O₃ equivalent to 30 ppmv ClO₂ and 175 ppmv O₃ equivalent to 70 ppmv ClO₂. Since 175 ppmv O₃ was not tested, the estimated log reduction was extrapolated from data from lower concentrations for comparison with ClO₂.

Table 7. Log reduction of *Escherichia coli* on stainless-steel coupons treated with ozone (75 and 175 ppmv) and chlorine dioxide (30 and 70 ppmv) at equivalent oxidative concentrations under low (66.8 ± 0.6%) and high (91.1 ± 1.3%) relative humidity (RH).

RH Level	75 ppmv O₃	30 ppmv ClO₂	125 ppmv O₃	70 ppmv ClO₂	175 ppmv O₃ extrapolated
66.8 ± 0.6% (Low RH)	0.9 ± 0.2 ^B	1.9 ± 0.5 ^B	1.2 ± 0.2 ^B	1.7 ± 0.4 ^B	1.5 ± 0.5
91.1 ± 1.3% (High RH)	2.0 ± 0.2 ^B	4.6 ± 0.6 ^A	3.6 ± 0.8 ^A	4.7 ± 0.3 ^A	4.8 ± 0.2

Different letters indicate statistically significant differences between relative humidity levels within each concentration at p < 0.05. Values are expressed as mean ± standard deviation (n = 3).

Chlorine dioxide showed higher log reductions than ozone under low relative humidity 66.8 ± 0.6%. Suggesting that even at lower concentrations and low humidity, chlorine dioxide still performed better. While at high relative humidities (91.1 ± 1.3%), both gases were effective at inactivating *E. coli*, but chlorine dioxide still managed to

produce a higher log reduction than ozone. Tukey's test confirmed that the difference in log reduction of *E. coli* between ClO₂ and O₃ at 91.1 % RH and the equivalent oxidative concentrations was statistically significance. Though, it should be noted that for chlorine dioxide measured at high relative humidity, the log reductions reached the maximum measurable limit. Therefore, the actual inactivation may have been even greater but could not be quantified.

Estimated D-values for Ozone Treatments

In **Table 8** the “estimated” D-values for inactivating *E. coli* that was inoculated onto stainless steel can be observed.

Table 8. Estimated D-values (minutes) for the log reduction of *Escherichia coli* on stainless steel coupons treated with ozone gas (15, 50, 75, 100, and 125 ppmv) under varying relative humidity levels ($66.8 \pm 0.6\%$, $75.3 \pm 1.1\%$, and $91.1 \pm 1.3\%$).

	D-value (minutes)				
RH Level	15 ppmv	50 ppmv	75 ppmv	100 ppmv	125 ppmv
66.8 ± 0.6% (Low RH)	N/A	N/A	33.0	28.4	24.8
75.3 ± 1.1% (Medium RH)	N/A	N/A	19.6	17.2	15.8
91.1 ± 1.3% (High RH)	52.4	19.1	15.0	11.0	8.4

N/A= Not available.

High relative humidities reduced the D-values, for example, at 75 ppmv the D-value decreased from 33.0 minutes at 66.8% RH to 15.0 minutes at 91.1% RH, and at 125 ppmv it dropped from 24.8 minutes at 66.8% RH to 8.4 minutes at 91.1% RH. This suggests that the time required for the inactivation was reduced by almost half in all concentrations.

Figure 5 shows the estimated log D-values of *E. coli* at different ozone concentrations and relative humidity levels. These are represented as “estimated” log D-values because they are calculated from log reduction data for treatment at 30 minutes only. Traditionally, D-values are obtained from a straight line fitted to log reductions at different treatment times.

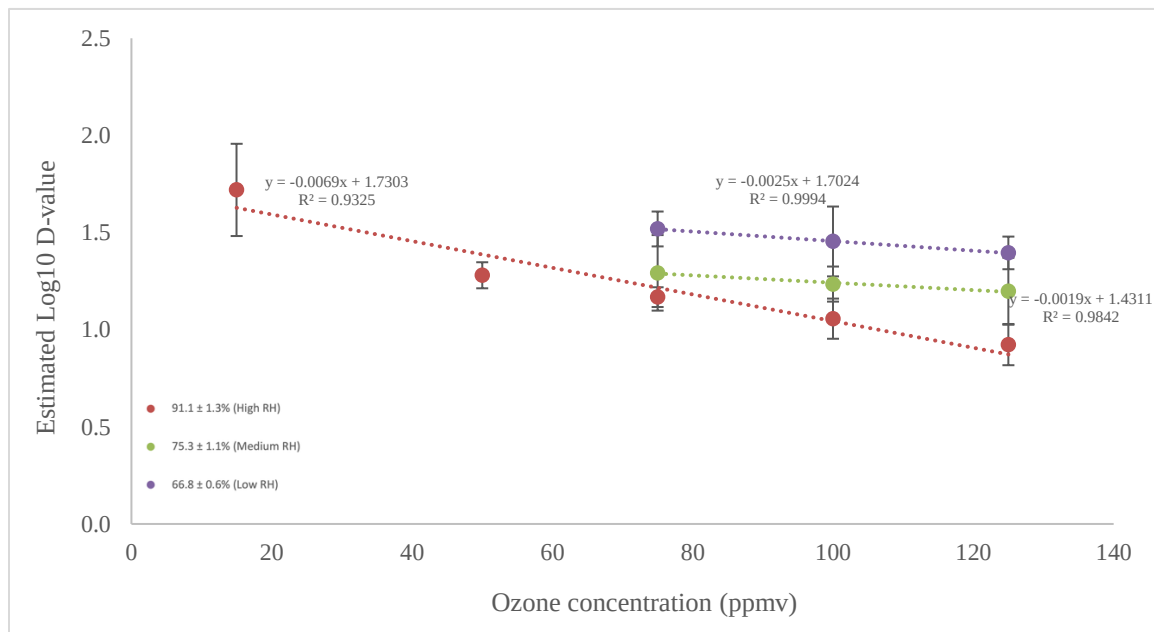


Figure 5. Estimated log D-values of *Escherichia coli* treated with ozone gas across different concentrations (15, 50, 75, 100, and 125 ppmv) under varying relative humidity conditions. Each line represents a relative humidity level (66.8 ± 0.6%, 75.3 ± 1.1%, 91.1 ± 1.3%), and Error bars represent mean estimated log D-value ± standard deviation (n = 3). Higher log D-values indicate lower inactivation efficiency and lower log D-values indicate higher microbial inactivation efficiency.

The estimated log D-values decreased as ozone concentration increased, this means that at 125 ppmv ozone level the time required to obtain a 1 log reduction was of ~8.4 minutes (having a log D-value of 0.92). And the highest log D-value was observed at 15 ppmv with a relative humidity of $91.1 \pm 1.3\%$ (log D-value of 1.72), indicating that the inactivation of *E. coli* was slower (~52.4 minutes). For the concentrations of 75, 100, and 125 ppmv when a relative humidity of $66.8 \pm 0.6\%$ was used the log D-values ranged from 1.4 (~24.8 minutes) and 1.5 (~33 minutes). These results show that treatments were effective in reducing *E. coli*, but achieving shorter inactivation times requires the combination of high relative humidity and high concentrations.

Discussion

Effect of ozone concentration on the inactivation of Escherichia coli under high relative humidity and Influence of Relative Humidity on the Log Reduction of Escherichia coli Using Gaseous Ozone

At high concentrations, ozone has more reactive molecules available to attack and destroy bacteria via damaging cell membranes, proteins, and nucleic acids (Xue et al., 2023). Relative humidity influenced the inactivation of *Escherichia coli* in this study. Treatments made at relative humidities of $66.8 \pm 0.6\%$ only achieved log reductions of 0.9-1.2 log cfu/coupon across concentrations, while treatments made at an intermediate relative humidity level of $75.3 \pm 1.1\%$ achieved log reductions of 1.5-1.9 log cfu/coupon. However, the highest log reduction occurred at the relative humidity of $91.1 \pm 1.3\%$ which yielded log reductions of 2.0-3.6 log cfu/coupon. These findings are consistent with previous research that showed that ozone concentration and treatment time improve

microbial inactivation (Emer et al., 2008). Emer et al. (2008) evaluated the log reduction of *E. coli* ATCC 8734 inoculated onto whole and ground black peppers and reported that after 30 minutes, using a concentration of 100 ppmv a 4 log reduction was obtained, but when they tested a concentration of 20 ppmv for 30 minutes resulted in only a 1 log reduction.

Similarly, M. Sharma and Hudson (2008) reported that they obtained a 3.1 log reduction of *Escherichia coli* ATCC 25922 inoculated onto sterile plastic trays (dry samples) when treated with 25 ppmv for 20 minutes at a relative humidity of 95%. High relative humidity improves surface moisture and promotes the formation of hydroxyl groups, which leads to a more effective microbial inactivation (Epelle, Emmerson, et al., 2022). In addition, Han et al. (2002) found that applying 2 mg/L (approximately 1,002 ppmv) for 25 minutes at 90% relative humidity resulted into a log reduction of 1.94 cfu/5g, but using 8 mg/L (approximately 4,007 ppmv) under same contact time and relative humidity, there was an increase of log reduction, reaching 7.35 logs cfu/5g. These studies demonstrate that high concentrations and high relative humidity levels result in higher inactivation rates.

High humidity contributes to microbial inactivation. Physically, using high humidity helps to re-hydrate cells, this allows for a thin water layer to form on the surface, which improves the contact between ozone and the pathogen, but, chemically speaking the moisture enables ozone to react with water in order to produce highly reactive radicals, that then attack and inactivate the pathogens through oxidative damage (STERISAFE®, 2025). Likewise, Grignani et al. (2021) reported in their study that high

humidity enhances ozone's reactivity by promoting the formation of hydroxyl radicals in the moist air.

Banerjee et al. (2024), evaluated the use of low concentration of gaseous ozone (0.1–5 ppmv) against *Escherichia coli* and other bacteria. They found that at humidities of > 70% ozone worked slightly better than low humidity (< 40%) and ambient humidity (40-60%), but it was not statistically significant. Similarly, the results from Cruz et al. (2025) showed a 3.7 log reduction from *E. coli* ATCC 25922 after a 30 minute exposure of 0.4-1.3 ppmv ozone in an air conditioned pilot scale chamber that they maintained at a relative humidity of 80%. These can be related to the results found on this study, since they show that while the ozone concentration is important to obtain a greater microbial log reduction, it is not the determinant factor since it also depends on exposure time and humidity.

Additionally, Aydogan and Gurol (2006) found that increasing the relative humidity from 70% to 95% resulted in reduced D-values from 107 minutes to 26 minutes at 10 mg/L (approximately 5,008.54 ppmv), and the authors attributed this change to the formation of moisture films and thus improving the solubility and diffusion of ozone on surfaces. In contrast, Epelle, Emmerson, et al. (2022) compared 2 ppmv and 4 ppmv for 8 minutes under a relative humidity of $50 \pm 2\%$. The 4 ppmv achieved a 4-log reduction of *Escherichia coli* NTCC1290 that was inoculated onto fabric swatches (35% cotton and 65% polyester), whereas 2 ppmv achieved a 3.5 log reduction. While this log reduction had such a low ozone concentration is unusual, this result demonstrates that even at low humidity it is possible to produce high log reduction. And, Marino et al. (2018)

demonstrated that applying 20 ppmv for 20 minutes under ambient humidity (40-60%), a log reduction of 4.72 was observed in the inactivation of *S. aureus* (on what surface?). Supporting this, in their research, Westover et al. (2022) they observed similar results when they used 20 ppmv of ozone gas for 30 minutes at room humidity to inactivate *E. coli* strain K12 that was inoculated onto well plates which resulted in a 1 log reduction, while when increasing treatment time to 120 minutes at the same concentration the reduction improved to a 2 log reduction.

Comparison of Ozone and Chlorine Dioxide at Equivalent Oxidative Concentrations

At equivalent oxidative concentrations, ozone and chlorine dioxide showed different behaviors when inactivating *Escherichia coli*. Chemically, ozone has a higher standard oxidation potential (2.07 V) than chlorine dioxide (0.95 V) which can be interpreted as ozone being the stronger oxidant, but chlorine dioxide's gas stability, reactivity, and persistence in moist air is what contributes to achieving high log reductions in surface when it is applied in practice. Since chlorine dioxide is more stable, it remains active longer when it is used in the gas phase, and this gas reacts through selective, single-electron transfer mechanisms rather than a broad, radical-driven oxidation (Gordon & Bubnis, 1999). This allows chlorine dioxide to diffuse and penetrate onto the hydrated surface layers more effectively than ozone, even when low concentrations are used.

Under high relative humidity ($91.1 \pm 1.3 \%$), chlorine dioxide achieved a reduction of almost 5 log cfu/coupon at both concentrations (30 ppmv and 70 ppmv) while ozone had a progressive increase in log reduction across concentration, from 2 log

cfu/coupon to 3.6 log to 4.8 log for 75 ppmv, 125 ppmv, and 175 ppmv, respectively. This suggests that chlorine dioxide can obtain a high log reduction even at low concentrations, whereas ozone needs higher concentrations to achieve the same results. However, when low relative humidity ($66.8 \pm 0.6\%$) was applied, the difference in log reduction between gases was minimal. The extrapolated results from 175 ppmv O_3 showed an average log reduction of 1.5 and the 70 ppmv ClO_2 (the equivalent oxidative concentration for 175 ppmv O_3) resulted in a 1.7 log reduction. The similarities in the results propose that when lower humidities are used in treatment the gases become less reactive due to the lack of moisture. Obinata (2022) indicated in their review that low concentrations are suitable when applied for surface or space decontamination due to its stability and reactivity.

These findings are consistent with previous studies reporting that chlorine dioxide maintains its disinfectant activity under humid conditions (by remaining as a dissolved gas in water) and achieves higher microbial reductions on food contact surfaces compared to ozone (Gómez-López et al., 2009). Park and Kang (2018) reported that chlorine dioxide gas under high humidity ($92 \pm 1\%$) resulted in a 2.37 to 3.03 log reduction of *E. coli* cells inoculated onto 304 stainless steel (SS) coupons with No.4 finish (5×2 cm) by treating the samples with 20 ppmv for 30 minutes. Likewise, Trinetta et al. (2013) showed that chlorine dioxide gas yielded a log reduction of 2.7, 2.9, and 3.6 on strawberries, cantaloupes, and roma tomatoes, respectively with a treatment period of 3 minutes combined with a concentration of 10 mg/L (approximately 3,562 ppmv).

Unlike the previous studies, Trinetta et al. (2011) reported greater reductions from gaseous ozone than with gaseous chlorine dioxide when they were compared on the

inactivation effect on *E. coli* O157:H7 (EDL 933) inoculated onto lettuce seeds.

Treatment with 10 mg/L of chlorine dioxide (approximately 3,622ppmv) had a contact time of 3 min under a relative humidity of 75%, this achieved a 1.94 log reduction. In contrast, exposure to 4.3 mg/L of ozone (approximately 2,190.43 ppmv) for 5 minutes obtained a log reduction of 3.53. Moreover, the study by Cho et al. (2010), where they compared mechanisms of inactivation of ozone, chlorine dioxide, and chlorine and expressed that the CT values (concentration x time) required to achieve a 1 log reduction varied among the disinfectants and was inversely proportional to the oxidation potential. Ozone required the lowest CT value of 0.0021 mg·min/L since it had the highest's oxidation potential (2.07 V). They stated that the oxidation potential for chlorine dioxide was 1.91 V thus resulting in a CT value of 0.030 mg·min/L, and lastly free chlorine which had the lowest oxidation potential (1.36 V) had the highest CT value of 0.085 mg·min/L.

From these findings, it can be inferred that while ozone has a higher oxidation potential that allows for a faster cell damage through a non-selective reaction, chlorine dioxide selective mechanism (attack on the proteins) and improved solubility helps it have an increased inactivation.

Estimated Log D-values for Ozone Treatments

The estimated log D-values indicates the time required to obtain a 1 log reduction using only a 30-minute treatment. At $91.1 \pm 1.3\%$ relative humidity and 125 ppmv ozone, the log D-value was about 0.9 (~8.4 minutes), which it can be compared to the log D-value of 1.5 (~33 minutes) at $66.8 \pm 0.6\%$ relative humidity. This can suggest that *E. coli*

required less time to be inactivated under high humidity. These results can be compared by the ones reported by Hamid et al. (2025), where they point out that it is often complicated to compare the results from studies because of the different ways of reporting the units. Due to this, they recommend using standardized metrics, such as D-values, since it would make it easier to compare ozone efficacy across various conditions. D-value results can estimate required exposure times based on targeted log reductions, ozone concentration, and humidity level.

Grignani et al. (2021) demonstrated that using ozone concentrations between 1 and 25 ppmv can achieve a 90% virus inactivation within 10-180 minutes depending on humidity and contact time. It was observed that relative humidities above > 70% improved the effect of ozone in inactivating viruses. In another research, the authors reported that after a treatment of 19.58 ppmv for 5 minutes under a relative humidity of 97% they obtained a log reduction of 1.18 of *E. coli* strain (ATCC 25922) (Cabral et al., 2024). This agrees with our estimated log D-values for *E. coli* that declined at 91.1 ± 1.3 % humidity, for this reason it can be suggested that under humid conditions ozone's oxidative efficiency increases.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

This study evaluated the effects of ozone and chlorine dioxide concentrations, as well as relative humidity, on the log reduction of *Escherichia coli* inoculated onto stainless steel surfaces. The results demonstrated that both factors significantly affected microbial inactivation. The highest log reduction occurred under high relative humidity ($91.1 \pm 1.3\%$), which highlights the importance of cell hydration in order to inactivate *E. coli*. Although the statistical analysis stated that the effect of ozone concentration on the inactivation of *E. coli* did not depend on relative humidity, both factors contributed independently to the inactivation. Overall, these findings confirm that gaseous ozone is an effective disinfectant/sanitizer and that to improve inactivation it is important to maintain humidity levels above $> 75\%$.

Gaseous ozone and chlorine dioxide both proved to be effective in reducing *E. coli* population on stainless steel coupons under two different humidity conditions ($91.1 \pm 1.3\%$ and $66.8 \pm 0.6\%$). In the experiments performed for this study, both gases achieved considerable log reductions, especially at a relative humidity of $91.1 \pm 1.3\%$. The comparison at equivalent oxidative concentrations suggest that chlorine dioxide may perform more effectively than ozone under similar conditions. Several factors may explain this difference, including the antimicrobial mechanism of each gas. Ozone acts broadly by targeting multiple cellular components, while chlorine dioxide selectively disrupts proteins, weakening the cell and enhancing susceptibility to inactivation.

Difference in gas solubility may also be able to explain why, regardless of concentration, chlorine dioxide obtained a higher log reduction compared to ozone under high humidity. Because chlorine dioxide is more soluble than ozone, this makes it possible for it to interact more effectively with hydrated cells, especially when the treatment chamber is already pre-conditioned, since that would allow for humidity and gas to become more uniform. Additionally, the decomposition rate of each gas can also influence how they work. Ozone is known to degrade fast (~ 20-minute half-life at room temperature in water), limiting its effective contact time. Whereas chlorine dioxide remains a dissolved gas in water and active for longer periods. But further research is needed to fully understand these mechanisms. The results confirm that both gases can achieve significant reductions in *E. coli*, ranging from 3.6 to 4.8 log (our upper limit of detection) making them highly effective disinfectants/sanitizers.

Future studies comparing ozone and chlorine dioxide at equivalent oxidative concentrations should extend the comparison beyond these concentrations by examining the kinetics of oxidant decay, the formation of reactive oxygen species (ROS), and the mechanisms responsible for microbial inactivation. It is also recommended to test shorter treatment times while maintaining high relative humidity levels, between 75% and 90%, and to explore a broader range of equivalence ratios.

Future studies on ozone gas treatments should assess the effect of post-treatment humidification to determine whether oxidative stress arises during the drying phase or from direct transfer to treatment conditions. Also measuring water activity at different

stages of the process may help explain how moisture availability influences microbial inactivation.

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VITA

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