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The application of ozone within the food industry, mode of action, current and future applications, and regulatory compliance

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Abstract

The food industry faces numerous challenges today, with the prevention and reduction of microbial contamination being a critical focus. While traditional chemical-based methods are effective and widely used, rising energy costs, the development of microbial tolerances, and growing awareness of the ecological impact of chemical biocides have renewed interest in novel biocides. Ozone, in both its gaseous and aqueous forms, is recognized as a potent disinfectant against bacteria, viruses, and fungi due to its high oxidation potential. Our review highlights several studies on the applications of ozone within the food industry, including its use for surface and aerosol disinfection and its capacity to reduce viable *Listeria monocytogenes*, a pertinent foodborne pathogen harbouring environmental and biocide stress tolerances and biofilm former. We also explore the use of ozone in food treatment and preservation, specifically on blueberries, apples, carrots, cabbage, and cherry tomatoes. While ozone is an effective disinfectant, it is important to consider material incompatibility, and the risks associated with prolonged human exposure to high concentrations. Nevertheless, for certain applications, ozone proves to be an efficacious and valuable alternative or complementary method for microbial control. Compliance with the biocide products regulation will require ozone device manufacturers to produce proven efficacy and safety data in line with British standards based on European standards (BS EN), and researchers to propose adaptations to account for ozone's unique properties.

Impact Statement

In this mini review, we evaluate the applications of both gaseous and aqueous ozone within the food industry. With the industry seeking alternative disinfection techniques to commonly used chemicals, ozone, a well-known and utilized oxidant, has been re-discovered and here we relay some current trends related to its use in food processing, surface and aerosol treatments, and biofilms. We also discuss some of the challenges associated with biocide products regulation compliance for both manufacturers, users, and researchers. We aim to address research gaps and provide information to end users on applications and safety.

Keywords: ozone; disinfection; food processing; regulatory; biocide products regulation

Introduction

The global food industry is continually challenged by persistent bacterial pathogens like *Escherichia coli*, *Salmonella spp*., and *Listeria monocytogenes*. which are known to survive, proliferate, and persist in ingredients (e.g. salads), food products themselves (ready to eat), and within food processing environments (EFSA Panel on Biological Hazards (BIOHAZ) et al. 2024). Traditional chemical disinfectants such as quaternary ammonium compounds and hydrogen peroxide are effective but are increasingly questioned due to concerns over microbial resistance, environmental sustainability, and cost (Ashraf et al. 2014, Curran et al. 2019). Ozone has gained attention as a promising alternative due to its ability to oxidize a wide range of microorganisms and its environmental benefits, such as *in-situ* production and lack of residual by-products (Xue et al. 2023). It is also worth considering the rise of antimicrobial resistance in healthcare as a warning against the persistent and overuse of chemical biocides within other industries. Research into bacteria-biocide tolerances is ongoing and a potential rise in tolerant microorganisms within food manufacturing environments (FMEs) is a subject of interest, especially when implicated within biofilms (Maillard 2018). This review provides a critical overview of ozone's applications in food safety, highlighting key gaps in the literature and outlining potential regulatory challenges.

Research into alternatives which can be used in place of, or in synergy with, conventional chemical biocides for a wide range of applications is a growing topic. This growth can be attributed to awareness of potential microbial tolerance and efficacy concerns, but also a wider awareness of sustainability through reducing the use of environmentally persistent chemicals, transportation, and overall cost (Ashraf et al. 2014, Raffo and Paoletti 2022). It should be noted that cleaning and disinfection operations are responsible for the greatest environmental impacts (water and energy consumption, wastewater, etc.) in a number of food processing plants (Pascual et al. 2007).

In contrast to conventional chemicals, ozone has an enhanced appeal within the food industry, this is due to its penetration ability within inaccessible areas and its relatively short half-life, where it returns to oxygen, reducing the likelihood of harmful residues being left on surfaces (Xue et al. 2023). It

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is also produced *in situ*, reducing the need for storage, transportation, and shelf-life considerations. Ozone use in disinfection has been demonstrated for the prevention and removal of pathogens in the food industry, with recorded applications for surface sanitization and the preservation of perishable goods such as meats, seafood, fruits, and vegetables (Arifianto et al. 2024, Monica et al. 2024).

Whilst ozone use within food processing is well documented (Kim et al. 2003, O'Donnell 2012, Brodowska et al. 2018, Epelle et al. 2023, İbanoğlu 2023), it is not a silver bullet in terms of microbial control. The workplace exposure limit in the UK of 0.2 parts per million (ppm) over 15 min, from our experience is potentially below that, which would have a significant impact on high concentrations of surface attached microorganisms. Ozone also has a high oxidation potential, and therefore, there are material compatibility concerns with the use of both gaseous and aqueous ozone, especially with rubber (Zheng et al. 2021) and potential effects on lipids within food products (Çelebi et al. 2024). There are also regulatory challenges related to biocide products regulation (BPR) compliance in the UK, which are focused on proving efficacy, safety and the identification of disinfection by-products. However, under certain conditions and for specific applications, ozone can be an effective disinfectant against bacteria, fungi, and viruses with a number of advantages over conventional chemical biocides such as *in-situ* production, non-specific biocide capabilities, and lack of harmful residues.

The adoption of ozone disinfection represents a forward-thinking approach to enhancing food safety and in turn reducing food waste. With growing interest and ongoing research, there is increasing potential for ozone use in further applications within air and surface disinfection as well as new research techniques to demonstrate its adherence to regulatory requirements.

Ozone formation and mode of action

Ozone (O_3, 48 g mol^{-1}) is a triatomic allotrope of oxygen that is less stable than its diatomic counterpart, oxygen gas (O_2, 32 g mol^{-1}) (Simpson 1947). Ozone is formed naturally in the stratosphere, where O_2 molecules undergo homolytic fission by solar ultraviolet (UV) radiation to form atomic oxygen radicals (Finlayson-Pitts and Pitts 1993). These radicals interact with further O_2 molecules to form ozone (Hatakeyama et al. 1987). Additionally, ozone can be formed by the electrical discharge from lighting, providing enough energy to split O_2 molecules and form ozone (Cleland and Galloway 2015). Both of these processes can be replicated *in-situ* by UV lamps (UV-radiation) and electrodes (corona discharge) to produce ozone for various applications such as surface/air disinfection, odour removal, and water purification.

The molecular structure of ozone facilitates the central oxygen atom to form a double bond with either one of its outside oxygens and a single bond with its third oxygen atom (Glockler and Matlack 1946). Due to the differing electronegative forces from unequal electron distribution, the geometry of ozone molecules forms at a bent angle of ∼116°, which deviates from the ideal tetrahedral angle of 109.5°, and contains varying bond lengths (O3: ∼1.278 Å) that are between single (O–O: ∼1.48 Å) and double bonds (O = O: ∼1.21 Å) (Glockler and Matlack 1946, Simpson 1947).

This strained molecular structure increases the repulsive force between the oxygen atoms, contributing to the unstable nature of ozone and making it prone to dissociation producing oxygen radicals and leading to its high oxidation potential and thus its application within disinfection (Yu et al. 1999, Matsumi and Kawasaki 2003, Xue et al. 2023).

With its high oxidising potential, microbial exposure to ozone generates reactive oxygen species (ROS) such as superoxide anions ($O2^{-}$), hydroperoxyl radicals ($HO_2^{\cdot}$), and hydroxyl radicals ($^{\cdot}OH$) (Atkinson and Carter 1984, Oh et al. 1999). Accumulation of these ROS in combination with ozone leads to oxidation of the cell wall and consequently cellular destruction through cell lysis (Li et al. 2020, Xue et al. 2023). Ozone also contributes to cellular death by partial or complete degradation of cellular components such as peptides, genetic material, and lipopolysaccharides (Li et al. 2020).

Oxidation of polyunsaturated fatty acids also results in conversion to peroxides which adds to the degradation of cellular envelopes (Feng et al. 2018). Ozone is applied for disinfection in both its gaseous and aqueous form, with studies demonstrating its efficacy for the successful inactivation of fungi, bacteria, viruses, and protozoa (Allen et al. 2003, Brié et al. 2018, Feng et al. 2018, Panebianco et al. 2021, Tizaoui et al. 2022, Ahortor et al. 2023). However, it is challenging for researchers to compare efficacy across studies due to variations in strains used, test method procedures, ozone concentrations, contact times, and humidity and temperature effects.

Ozone applications within the food industry

Surface disinfection

For surface disinfection, ozone is typically applied as a gas within a closed environment. The concentration used depends on the required level of disinfection: higher concentrations can be more effective but take longer to generate, achieve adequate distribution, and remove. Ozone's versatility allows for small scale applications such as a benchtop closed system, or on a larger scale in food processing rooms. Within the food industry, ozone can be used as an alternative to other biocides, with advantages over certain disinfectants, such as quaternary ammonium compounds that can leave unwanted residues on surfaces following use or complementary to other biocides, to create a synergistic effect as a form of hurdle technology (Zoutman et al. 2011, Condell et al. 2012, Meade et al. 2021, Botondi et al. 2023, Coombs et al. 2023, Xue et al. 2023).

Several intrinsic and extrinsic factors influence the efficacy of gaseous ozone disinfection. Intrinsic factors include the type of surface targeted (Wood et al. 2020, Zucker et al. 2021), the microbial strain (Akbas and Ozdemir 2008, Wood et al. 2020, Ali and Abdallah 2022), their physiological states (Wani et al. 2016), and inoculation volume and concentration (Epelle et al. 2023). Extrinsic factors include relative humidity (Blanchard et al. 2020, Tizaoui et al. 2022), ozone concentration (Marino et al. 2018, Piletić et al. 2022)[6], ozone contact time (Uppal et al. 2021, Epelle et al. 2022), and temperature (Blanchard et al. 2020, Thill and Spaltenstein 2020)

Megahed et al. (2018), investigated the use of gaseous ozone to disinfect cattle manure-based pathogens on various materials commonly used in the dairy industry, such as plastic, metal, nylon, rubber, and wood. After an 8-min exposure at an ozone concentration of 9 ppm, smooth surfaces (plastic and metal) observed a 3.2 and 1.6 log reduction, respectively, while rougher surfaces (wood, nylon, and rubber) averaged a 0.9 log reduction. Marino et al. (2024) reported a 5.23 log

CFU cm^{-2} reduction of *Pseudomonas fluorescens* biofilms on stainless steel after 60 min of exposure to 20 ppm of gaseous ozone, whilst Piletic et al. (2022) showed that an hour exposure to 25 ppm ozone resulted in a 2–2.5 log CFU cm^{-2} reduction in *Klebsiella pneumoniae* biofilms on ceramic tiles. In contrast, Serra et al. (2003) showed that treatment of a cheese ripening room with gaseous ozone (produced at 8 g h^{-1}, 12 h per day weeks 1–7, 40 min per hour weeks 8–20) did not result in significant reduction of moulds on surfaces. This highlights the differences in ozone efficacy against different microorganisms in different settings and perhaps suggests that moulds are more tolerant compared to bacteria.

Efficacy against *Listeria* spp.

Listeria spp. harbour considerable tolerances to environmental stressors encountered in the FME, including high and low temperatures, as well as acid and osmotic stress (Wiktorczyk-Kapischke et al. 2021). As a result, *Listeria* spp. serves as both a model organism for the exploration of ozone and as a pressing motivator for novel disinfectant technologies.

The *in vitro* effect of gaseous ozone on *Listeria* spp. on abiotic and biotic surfaces, with and without established biofilms, suggest that pre-established biofilms hinder the disinfectant efficacy of ozone (Panebianco et al. 2021). Comparatively, ozone against *Listeria* spp. without biofilms demonstrates a greater capacity for the inactivation of planktonic and newly adhered cells (de Candia et al. 2015, Wani et al. 2015, Panebianco et al. 2021).

Panebianco and colleagues et al. (2021) investigated ozone efficacy at 50 ppm, ≥90%RH over a period of 10 min, 30 min, and 6 h for planktonic cells inoculated on agar plates and 6 h for biofilms on polystyrene tissue culture plates. Within 10 min, a 3.7 ± 0.4 log10 CFU ml^{-1} reduction was observed followed by 3.9 ± 0.4 log10 CFU ml^{-1} reduction by 30 min for planktonic cells. Total inactivation over 6 h was observed in 17 of 22 strains, with over a 7 log10 CFU ml^{-1} reduction observed in the remaining five for planktonic cells. For biofilms, 0.7 ± 0.4 log CFU ml^{-1} and 0.8 ± 0.5 log CFU ml^{-1} reductions were observed in inhibition (ozone exposure prior to biofilm formation) and eradication (ozone exposure after biofilm formation) treatments, respectively (Panebianco et al. 2021). Contrastingly, Harada and Nascimento (2021) used 45 ± 2 ppm ozone to reduce *L. monocytogenes* recovery to below detectable limits within 5 min of exposure in biofilms on polypropylene. This incongruence requires further exploration, given the prevalence of biofilms within the FME (Marino et al. 2018).

On food surfaces, concentrations of gaseous ozone as low as 1 ppm have demonstrated a 1 log10 CFU ml^{-1} reduction against *L. innocua* inoculated onto spinach (Wani et al. 2015).

The use of gaseous ozone to reduce *Listeria* spp. has also been studied on beef, where incubation at 4°C and 280 g O_3 m^{-3} (142 ppm) for up to 16 days resulted in a ≥4 log CFU/g reduction compared to storage at 4°C and 10°C (Giménez et al. 2021). Concentrations of 78.7 μg l^{-1} (40 ppm) O_3 against *L. innocua* on the surface of apples resulted in a >3 log10 CFU/apple reduction compared to controlled atmosphere and refrigerated air conditions over 36 weeks of storage (Shen et al. 2021).

On the surface of blueberries, *L. monocytogenes* exposed to 2−4 ppm for 10 days demonstrated a >3 log10 CFU ml^{-1} reduction compared to control groups (Concha-Meyer et al. 2014). Conversely, Panebianco et al. (2022) identified that the use of gaseous ozone against food surface flora may inhibit *Listeria* spp. antagonists such as lactobacilli, potentially enhancing survival of *Listeria* spp. Exposing Gorgonzola rinds, inoculated with *L. monocytogenes*, to 2−4 ppm O_3 for 10 mins before storing the rind at 4°C for 28 days resulted in 1 log CFU g^{-1} increase of *L. monocytogenes*, followed by a decreasing population for the full 63-day duration. The findings of these studies indicate a varied but promising capacity of ozone to reduce *Listeria* load on surfaces, including against attached cells.

Aqueous ozone

Aqueous ozone is typically produced by dissolving gaseous ozone into a solution (Batagoda et al. 2019). On stainless steel discs, which simulate food industry surfaces, a concentration of 0.5 mg l^{-1} of static aqueous ozone resulted in a 1.82 log reduction of *P. fluorescens* after 10 min of exposure, whilst a dynamic exposure led to a 3.81 log reduction (Marino et al. 2018). For biofilms of *P. fluorescens*, static exposure achieved a 1.56 log reduction. While dynamic exposure achieved a 3.35 log reduction, indicating that dynamic application may be more effective (Marino et al. 2018). Soaking galvanized metal and polypropylene plastic in a manure mixture followed by 20 ml of aqueous ozone at 2 ppm for 4 min resulted in a 3.10 log reduction of manure-based pathogens, including *Bacillus cereus* and *E. coli*, on plastic and a 2.8 log reduction on metal (Megahed et al. 2018). The difference in results between the two surfaces could be explained by variations in the surface smoothness, with smoother surfaces (in this case, the plastic surface) allowing a greater ozone mass transfer and therefore more efficient ozone diffusion to the pathogens (Megahed et al. 2018).

Employing aqueous ozone to fruits and vegetables can effectively reduce pathogenic and spoilage microorganisms on their surfaces, thus extending their shelf life (Sarron et al. 2021, Xue et al. 2023). Batch washing carrots with 5 ppm of aqueous ozone resulted in a 1.56 log reduction of *L. monocytogenes*, while using it in a spray form achieved a 2.49 log reduction (Chandran et al. 2023). The increased efficacy when ozone was applied as a spray could be due to the effect of the water pressure from the spray assisting in the removal of bacteria from the surface (Chandran et al. 2023). Treating fresh-cut cabbage leaves with 1.4 mg l^{-1} of aqueous ozone for 10 min initially reduced aerobic bacteria by 3 log after washing, and inhibited bacterial growth by 1.6 log after 12 days of storage (Liu et al. 2020). Significant reductions in yeasts and moulds were also observed over 12 days, with coliforms only reducing by 0.8 log (Liu et al. 2020). Additionally, treating an onion with aqueous ozone provided an extended shelf life of 14.3%, 2 days, longer than a water washed onion (Aslam et al. 2021). These findings suggest that aqueous ozone could be used as an effective disinfectant for surfaces with multiple applications in the food industry.

Consideration must be given to the potential impacts of ozone on food quality (Akbas and Olmez 2007, Chauhan et al. 2011, Aslam et al. 2021). Aqueous ozone treatment, 1.4 mg l^{-1}, can negatively affect the antioxidant content of vegetables; cabbages treated with ozone showed a significantly lower antioxidant capacity, measured with a ferric reducing antioxidant power (FRAP) assay, than water washed ones after 12 days of storage (Liu et al. 2020). Ozone treatment can lead to the degradation of phenolics (Chauhan et al. 2011), an important natural antioxidant in carrots (Kumar and Goel 2019). Conversely, Taiye Mustapha et al. (2020) demonstrated that washing cherry tomatoes with aqueous ozone did not negatively impact their physicochemical properties, including loss of firmness and pH, bioactive compounds, such as the total phenolic and flavonoid contents, or total antioxidant activity, as measured by FRAP assays, after 21 days. Although, this was conducted at a concentration of 0.85 mg l^{-1} which may have a negative effect on microbial reduction. Hurdle technology can be applied to mitigate the adverse effects of ozone on food quality (Xue et al. 2023). A combination of 9 mg l^{-1} aqueous ozone and 2.5 ml l^{-1} lactic acid demonstrated an improved effectiveness in removing contaminants, such as *E. coli*, from fresh vegetables like tomatoes and carrots, without significantly altering the sensory qualities, such as colour and firmness, of the treated samples in comparison to the untreated samples (Pounraj et al. 2020).

Ozone can also be used in clean in place (CIP) systems, commonly used in processing equipment without the need for disassembly, ensuring rapid, repeatable cleaning of closed systems to prevent contamination (Avila-Sierra et al. 2021). Traditional CIP involves flushing pipelines with disinfectants, which can be costly and can be impactful on the environment (Englezos et al. 2019). Employing aqueous ozone can reduce operational, chemical, and sewage costs, leaving no residues, unlike chemical-based methods, although care should be given with regards to material compatibility (Canut and Pascual 2007).

In the wine industry, aqueous ozone has proven effective as a CIP agent. A 3.50 mg l^{-1} aqueous ozone treatment, for 30 min, achieved higher microbial reductions than peracetic acid (1%) in a bottling machine and pipelines, though it was less effective than peracetic acid against *Saccharomyces cerevisiae* (Englezos et al. 2019). This could indicate that ozone may be more applicable in targeting some microorganisms more than others. A pilot study simulating food processing equipment of water tanks and surrounding vessels showed that 10 ppm aqueous ozone for 10 min could remove biofilms of *P. fluorescens* below levels of detection, surpassing an alkaline cleaning soliton that left 1.4 and 1.5 log CFU ml^{-1} on the smooth and rough surfaces of the system respectively (Tirpanci Sivri et al. 2023). Additionally, using ozone in a bath substrate flow device, to simulate industrial CIP systems, enhanced the removal of starch from surfaces in the system (Avila-Sierra et al. 2020). The use of ozone also resulted in the degradation of starch, reducing contamination of wastewater after cleaning, combining these two processes in a single step (Avila-Sierra et al. 2020).

However, limitations exist for using ozone in CIP systems, especially with regards to the production of off-gases which need to be monitored and exposure controlled (Botondi et al. 2023). Furthermore, the interaction between ozone and the systems materials needs to be considered. Ozone, after repeated use, can lead to corrosion of certain materials, such as carbon steel (Sato et al. 1982, Panebianco et al. 2021). CIP systems using aqueous ozone would need to ensure that the equipment and systems are optimised for ozone use.

Biofilm prevention and remediation

The use of gaseous or aqueous ozone for the removal or inhibition of biofilms in the food industry and neighbouring industries has been the subject of several studies highlighting the potential for biofilm removal, though the distinction between wet surface biofilms and dry surface biofilms is not yet made (Marino et al. 2018, Shelobolina et al. 2018, Harada and Nascimento 2021, Panebianco et al. 2022).

Panebianco et al. (2021) hypothesized that the use of ozone in a preventative manner may limit the capacity of *L. monocytogenes* to form biofilms. However, in both preventative and eradicative treatments, only 27% and 32% of strains demonstrated a reduced biofilm production index compared to untreated groups. Another study examined the inactivation of biofilm of *P. fluorescens*, *S. aureus*, and *L. monocytogenes* (Marino et al. 2018). Against mono-species, multi-strain biofilms grown over 48 h, aqueous ozone of 0.5 ppm was able to inactivate between 4.47 and 3.29 log10 CFU/cm^{-2} of inoculated cells under dynamic conditions. This efficacy was reduced in static conditions to between 3.94 and 2.25 log10 CFU/cm^{-2}. Dynamic conditions outperformed static conditions for *P. fluorescens* and *S. aureus*, but not for *L. monocytogenes* where both conditions incurred similar results. In the same study, gaseous ozone performed in a both time and concentration dependent manner where a 60-minute exposure to 2 ppm resulted in between a 2.07 and 5.33 log10 CFU/cm^2 reduction. Gaseous ozone at 5 and 20 ppm for 30 and 20 min, respectively, were sufficient to render *S. aureus* undetectable. *Listeria monocytogenes* biofilms were particularly susceptible, being undetectable after 2 min of exposure at 2 ppm.

The efficacy of ozone against polymicrobial biofilms is also an area of pertinence, given the unlikelihood of mono-species biofilms outside of *in vitro* experimentation. This may be overseen by the undertaking of *in situ*, applied studies.

Aerosol disinfection

Growing attention and awareness across different industries has also been paid to bioaerosols, which are aerosols that contain particles of biological origin, including bacteria, viruses, fungi, and spores and as such can provide a route of disease transmission and food contamination, along with other chronic ill-health effects (Kim et al. 2018, Masotti et al. 2019a).

Within the food industry, microorganisms can become aerosolized in various environments such as processing facilities, poultry houses, and slaughterhouses via several routes, including food washing/processing procedures and cleaning processes within the facilities, which may pose a risk for contamination and occupational health (Theisinger and de Smidt 2017, Masotti et al. 2019a).

The use of ozone to disinfect airborne microorganisms has been studied in several cheese ripening/storage facilities (Serra et al. 2003, Pinto et al. 2007, Masotti et al. 2019b). Serra et al. (2003) showed that daily treatment with ozone (either 4 or 8 g h^{-1}) over a 3-month period (either 12 h overnight or 40 min of every hour) resulted in an approximate 10-fold decrease in the number of viable airborne mould colonies compared to the untreated control.

A more recent study reported on the use of ozone to reduce the number of culturable bacteria and yeasts within a packing room within a cheese ripening facility (Masotti et al. 2019b). In this study, it was shown that treatment with ozone (generated at a constant rate of 40l min^{-1}, predicted to be 5 ppm) for a total period of 3 hours overnight was shown to reduce the number of bacteria to 0 from 486 colonies and reduce the airborne load of mould and yeast by 99.7% and 99%, respectively (Masotti et al. 2019b).

A study by Jiang et al. (2018) which examined the efficacy of different disinfectants (including gaseous ozone) at reducing the number of airborne aerobic bacteria within broiler houses within the poultry industry. In this study, it was shown that treatment with ozone (100 g h^{-1} for 1 h) resulted in a reduction in the number of aerobic bacteria present from approximately 2200 cfu m^{-3} in untreated samples to approximately 200 cfu m^{-3} after treatment, it was more efficacious than treatment with available chlorine (reduction to 767 cfu m^{-3}), however, it was less effective than quaternary ammonium salt, glutaraldehyde, and a mixed disinfectant (aldehydes, quaternary ammonium salt, and alcohol) which showed the best efficacy, reducing the number of bacteria present to 22 cfu m^{-3}, overall indicating that ozone may provide some benefit in this context, although future studies could further investigate and shed more light on this potential application (Jiang et al. 2018).

Novel applications

Low to high care transfer

The use of ozone for the purpose of reducing contamination through low to high-risk transfer has yet to be explored *in situ*. However, the use of gaseous and aqueous ozone as a disinfectant in the FME has been the subject of research. This research has focused on food contact surface hygiene, food equipment disinfection, reuse of wastewater and lowering biological and chemical oxygen demand (Guzel-Seydim et al. 2004).

Ozone can also be used for the treatment of equipment or materials before transporting into high-risk zones. The use of both gaseous and aqueous ozone described above can be applied in material transfer hatches (MTHs). Though literature in the use here is currently limited, research by Qiao et al. (2022) explored the use of UV and gaseous ozone in the treatment of equipment in an MTH in an animal research facility. Therein, ozone and UV used synergistically outperformed control groups and indirect UV in reducing bacterial CFU. The use of ozone as a surface disinfectant has been discussed in previous sections, showing promise on materials such as plastic and metal (Megahed et al. 2018)

Regulatory compliance

Since 2013, ozone has been regulated as an ‘active substance’ under the Biocidal Products Regulation (BPR) within the European Union (EU). Following these changes, the BPR mandates that all ozone-based devices must be authorized as approved active-substances to enter commercial markets. Compliance with BPR is critical for manufacturers, as it ensures that only safe and effective products are available within commercial market. The authorization process driven by the European Chemicals Agency (ECHA), involves a thorough evaluation of product safety, efficacy, and environmental impact when implementing compliance to BPR. Manufacturers of ozone-based biocides must provide comprehensive data demonstrating efficacy against target microorganisms and ensuring it poses no risks to human health or the environment when used as intended.

To demonstrate compliance with health and safety guidelines, adherence to specific test methodologies, such as BS EN 17272:2020 and BS EN 13697:2015, is essential. These standards define crucial testing conditions, including biocide concentration, exposure times, and environmental parameters, to accurately assess the effectiveness of ozone and ensure consistency across testing protocols. Specifically, EN 17272 is used to evaluate the efficacy of gaseous ozone as an airborne disinfectant, while EN 13697 is used to assess the biocidal properties of aqueous ozone. Adherence to these standardized tests provides a validated framework that assures manufacturers and users of the reliability of ozone disinfection systems, facilitating compliance with stringent health and safety guidelines. This is crucial in sectors like the food industry, where maintaining stringent microbial control is paramount.

Despite these established standards, ozone possesses unique properties that may not be fully addressed by existing testing protocols, or in some cases, there may be no current standard for specific applications. Therefore, ongoing research is critical to expand and adapt these standards. Researchers play a pivotal role in conducting studies that modify and enhance existing methodologies to incorporate the distinctive characteristics and applications of ozone. On going work between our research group and the European Ozone Trade Association is actively engaged in adapting, developing and proposing new testing protocols to evaluate ozone’s efficacy in disinfecting bioaerosols and in the prevention and treatment of prominent biofilms in various industrial contexts. This collaborative effort between researchers and manufacturers ensures that ozone disinfection technologies continue to meet high standards of safety and efficacy. Furthermore, methods developed within such collaborations are reviewed by European regulating bodies to ensure adherence to BRP guidelines.

While ozone’s potential as a biocidal agent is becoming more established, it is important to note that its use as a plant protection product or pesticide, in the UK at least, is currently not permitted under the BPR. This restriction highlights the need for further research and regulatory consideration to explore the safe and effective use of ozone in further agricultural applications and to reduce reliance on other conventional chemicals. The rigorous assessment required for authorization under the BPR underscores the complexity of integrating ozone into broader biocidal and pesticidal frameworks. As ongoing research continues to investigate ozone’s diverse capabilities, it is crucial to address these regulatory challenges and ensure that any potential new applications meet the stringent safety and environmental standards established by European authorities. This ongoing dialogue between research institutions, industry stakeholders, and regulatory bodies will be essential for unlocking the full potential of ozone-based technologies in a safe and controlled manner.

Ozone shows significant promise as an alternative to conventional disinfection methods in the food industry. Its ability to disinfect without leaving harmful residues, combined with its effectiveness against key foodborne pathogens, makes it an attractive option. However, challenges remain in terms of regulatory compliance, material compatibility, and ensuring efficacy in large-scale operations. Ongoing research, including collaborations between industry and regulatory bodies, is essential to address these issues and unlock ozone’s full potential in ensuring food safety.

With a growing desire within the food industry to move towards other disinfection methods whilst maintaining high levels of microbial disinfection, ozone offers a useful alternative to traditional methods. Indeed, ozone in both its gaseous and aqueous forms has demonstrated efficacy against a range of microorganisms including the prominent foodborne pathogen *L. monocytogenes* as discussed throughout this review. Ozone disinfection has been utilized within a broad range of applications across the food industry including surface disinfection, food washing, beverage treatment and CIP procedures. However, there is still significant scope for further use within the industry, for example within airborne disinfection and against polymicrobial biofilms, with further research within these areas required. It is important however, to consider that ozone is not a catch-all solution and there are considerations such as occupational exposure hazards, potential effects on product quality and multiple factors which affect efficacy. Additionally, with changes to regulation that mean ozone devices must be compliant to the BPR, continued work to adapt the test methodologies to allow ozone devices to meet these standards will be paramount for continued and increased use of ozone in a safe and effective manner within the food industry.

Author contributions

Zak Hamid (Conceptualization [equal], Investigation [equal], Project administration [equal], Visualization [equal], Writing – original draft [lead], Writing – review & editing [equal]), Ben K. Meyrick (Writing – original draft [equal], Writing – review & editing [equal]), Joshua Macleod (Conceptualization [equal], Methodology [equal], Project administration [equal], Visualization [equal], Writing – original draft [equal], Writing – review & editing [equal]), Emily A. Heath (Project administration [equal], Writing – original draft [equal], Writing – review & editing [equal]), and James Blaxland (Conceptualization [equal], Funding acquisition [lead], Investigation [equal], Methodology [equal], Project administration [equal], Visualization [equal], Writing – original draft [equal], Writing – review & editing [equal])

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Data availability

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