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A B S T R A C T

Ozone treatment is an eco-friendly and cost-effective approach to achieve material disinfection, and this disinfection method is of utmost importance in the present global pandemic. The efficacy of ozone's oxidative potential on common microorganisms has been extensively studied, particularly in the food and water treatment industries. However, little is still understood regarding its antimicrobial capabilities for the treatment of textile substrates in air. In this study, fabric swatches inoculated with bacterial and fungal suspensions are exposed to ozone for different durations and at different ozone concentrations. Pathogenic bacteria (*Escherichia coli*, *Staphylococcus aureus*), and fungi (*Aspergillus fumigatus*, and *Candida albicans*), are the microbes utilised in this study. The efficacy of ozone is demonstrated by the complete removal of microbiota on the tested swatches when a concentration and exposure duration of 20 ppm and 4 mins are respectively maintained in a test ozone chamber. We expect the insights from this work to guide the development of new ozonation techniques capable of rapid sterilisation in industrial & public settings.

1. Introduction

The horrific impact of the Covid-19 pandemic on virtually all aspects of human life has led to growing global concerns on public health and safety (Cristiano, 2020). This has necessitated recent advances in the development of robust disinfection technologies capable of destroying harmful bacteria, fungi and viruses; some of these include steam autoclaving, ionizing radiation, and oxidative agents such as ethylene oxide and hydrogen peroxide (Chidambaranathan and Balasubramanium, 2019). Ozonation is a highly potent method, capable of destroying microorganisms (via oxidation) in both air and water (Magbanua Jr et al., 2006; Hayes et al., 2013; Ouf et al., 2016; Pagès et al., 2020; Ayed et al., 2021; de Almeida Kumlien et al., 2021). Its environmentally friendly characteristics (rapid auto-decomposition to oxygen) has also made its application suitable for a very wide range of industries (food, healthcare, aquaculture, waste-water treatment and textile) (Remondino and Valdenassi, 2018).

The use of ozone in the food industry is a widely studied subject with numerous research contributions published over the past 2 decades

(Guzel-Seydim et al. 2004; Rosenblum et al., 2012). Recent developments in the food industry have demonstrated the efficacy of ozone when used with other food-processing methods such as ultraviolet irradiation, washing with electrolysed water and modified atmosphere packaging (Klockow and Keener, 2009; Steffen et al., 2010; Patil and Bourke, 2012). In healthcare, ozone has been applied for medical instrument sterilisation, particularly for heat-sensitive materials (Luqueta et al., 2017). Sousa et al. (2011) carried out a comprehensive review in this field and concluded that ozone is a promising method for sterilizing medical devices; however, it is still in its initial phases of investigation for this purpose. Furthermore, recirculating aquaculture systems benefit immensely from ozone's application via the removal of algae, organic carbon, colour and odour (Summerfelt et al., 2009). Gonçalves and Gagnon (2011) further pointed out that the effectiveness of ozone treatment in aquaculture systems depends on exposure duration, ozone concentration, pathogen loads and levels of organic matter. The use of ozone for waste-water treatment represents one of its earliest industrial applications for similar purposes as with aquacultural systems. Compared to chlorine, which produces chlorinated organics (which are

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harmful to the environment), the use of ozone for water treatment prevents this problem; thus making it a friendlier alternative (Rice, 1996). The high activity of ozone across the whole pH range compensates for pH adjustment chemicals required in other water treatment procedures – another favourable property of aqueous ozone treatment (Eren et al., 2020).

In textiles, ozone has been shown to be effective in denim applications, cotton pre-treatment, dyeing, finishing and clearing and colour removal for different types of textile fibres (Rice et al., 2009; Körlü, 2018; Neral, 2018; Eren et al., 2020; Ben Fraj and Jaouachi, 2021). Despite these contributions, microbial inactivation by ozone on textile substrates is still an open research question. These organisms have varying sensitivity and resistance levels to ozone. Moulds are generally more resistant than yeasts; which are in turn more resistant than bacteria (Patil and Bourke, 2012). Viruses show similar resistance levels as bacteria to ozone, whereas, ozone has been shown to be less effective against fungal and bacterial spores than vegetative cells (Moore et al., 2000; Khadre et al., 2001). None of these studies has considered the interaction of ozone with these organisms, on textile substrates. Furthermore, an analysis of the interdependence between the ozone concentration employed and the exposure time is lacking in the literature, for different classes of organisms. These limitations are addressed in this study using a gram-positive bacteria (*Staphylococcus aureus - SA*), a gram-negative bacteria (*Escherichia coli - EC*), a primitive fungus (*Candida albicans - CA*), and a fully evolved fungus (*Aspergillus fumigatus - AF*). It is also worth mentioning that most industrial and large-scale deployments of ozone disinfection systems are plagued by very long cycle times. Based on the findings of this study, we provide some recommendations regarding possible improvement routes that could be pursued for large-scale disinfection purposes. The assurance of long-term substrate sterility as demonstrated herein, also strongly suggests the applicability of this sterilisation technique for personal protective equipment (PPE) in healthcare and clothing applications; thus paving the way for their reusability and reduced environmental impact.

2. Methodology

2.1. Ozone chamber

A 2-dimensional representation of the ozonation chamber (0.60 m × 0.60 m × 0.57 m) utilised for our study is shown in Fig. 1. The chamber consists of 4 ozone-generating (OG) low-pressure mercury ultraviolet (UV) lamps, which are 30 cm in length and 0.9 cm in diameter (Jelight Company Inc. USA). These lamps are an effective source of the 185 nm spectral line, which is absorbed by oxygen molecules in the chamber, for ozone production (air is used as the medium). These lamps also emit UV light at other wavelengths (as shown in Fig. 2). Furthermore, 2 ozone-free (OF) lamps are installed in the chamber, for ozone destruction, as well as for disinfection purposes. They are similar to the OG lamps but contain a doped quartz envelope that absorbs the 185 nm photons; preventing the formation of ozone. A warm-up time of less than 2 mins is usually required for optimal performance of the lamps (Fig. 2a). Preliminary tests show that ozone concentrations of 11 ppm, 20 ppm, 28 ppm and 32 ppm can be obtained in 4 mins, when 1, 2, 3, and 4 lamps are switched on, respectively. Ambient conditions were 20 °C and 50% relative humidity. The chamber is also equipped with a rotatable anchor (2.5 rpm), for vertically hanging and rotating the fabric swatches used (Table 1); this enables efficient surface area exposure for ozone's action. Given ozone's high relative density in air (≈ 1.8), an axial circulation fan (45 m^3/h) is inevitable for adequate gas circulation and is installed in the chamber.

Upon completing the disinfection cycle, a suction fan (with a variable speed controller), is utilised to pull the gas out of the chamber into a destruction unit, which is thereafter sent to the fume cupboard. A combination of the OF UV lamps and the centrifugal suction fan speeds up the process. The performance of the suction fan at different circulation speeds is subsequently described (Fig. 12). The ozone concentration is measured using 2 methods – probe attached to a monitor (Bosean Ltd. China) and via remote sensing of the gas (WinSensors Ltd. China). A control panel (Belmos Electrical Services, UK), couples all electrical components within the box, allowing a systematic variation of different parameters affecting the system, such as the number of lamps switched on, the suction rate of the centrifugal fan, and the rotation of the swatches.

2.2. Disinfection cycle

It is worth defining what the exposure/cycle time utilised in this study is. A typical ozone disinfection cycle in this study usually consists of the generation phase, a stabilisation phase and a decomposition phase. In the experiments performed herein, we define the cycle time as the duration of exposure of the substrate at the specifically desired ozone

1. Fume cupboard
2. Pressure gauge
3. Ozone monitor
4. Probe of ozone monitor
5. Inlet valve
6. 254 nm Ozone-free/ destroying (UVC) lamp
7. 185 nm + 254 nm (VUV + UVC) Ozone-generating lamps
8. Rotatable anchor
9. Fabric swatches
10. Remote ozone sensor
11. Ozone circulation fan
12. Ozone suction fan
13. Adjustable shelf
14. Temperature and humidity probe with monitor
15. Data acquisition system
16. Catalytic destruction unit
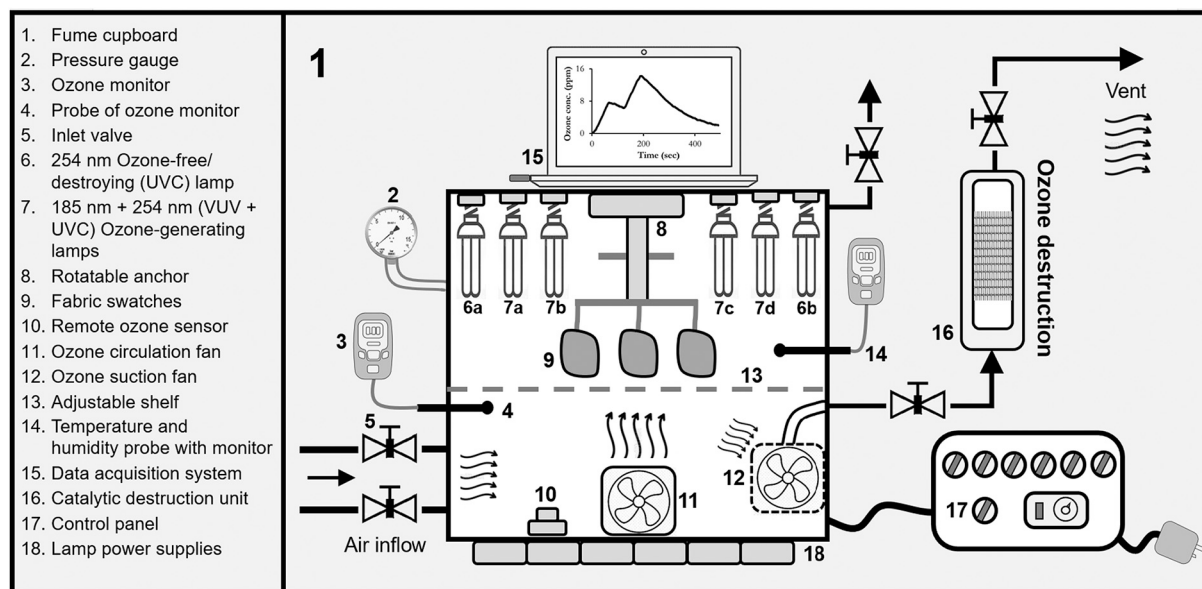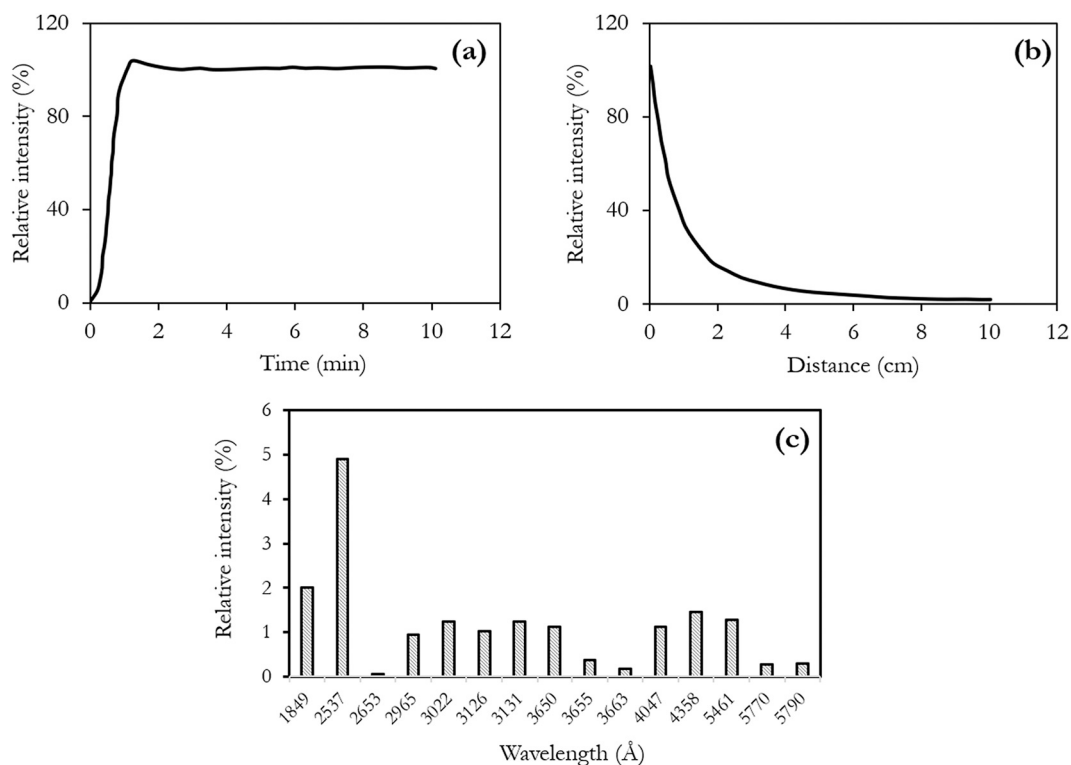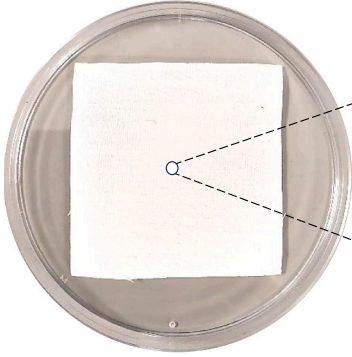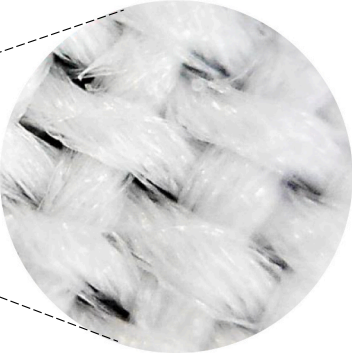17. Control panel
18. Lamp power supplies

Fig. 1. Experimental set-up for ozone disinfection.

Fig. 2. Ozone producing lamp specifications showing: (a) stabilisation time from start-up; this time is independent of the number of lamps and corresponds to the time it takes before the production of ozone can be observed in the chamber (b) relative intensity as a function of distance and (c) wavelength spectrogram (Jelight Company Inc., 2021).

Table 1
Micrographic image and material composition of fabric swatches applied.

Fabric swatch	Micrographic image of the fabric's weave structure (_____ 500 μm)
Material composition of fabric swatch utilised in this study: 65% Polyester; 35% cotton.	
Pre-treatment conditions: Autoclave sterilization after treatment with 70% ethanol solution.	
Approximate yarn number: 110 ± 5	

The micrographic image was obtained using an optical microscope. It is worth mentioning that only 1 fabric type was utilised in this study, as we were mainly interested in finding the optimal combination of ozone concentration and exposure time for effective decontamination. The impact of different fibre morphology/weave structure is a separate subject of ongoing investigation by the authors.

concentration. In essence, our definition of the cycle time is based on the stabilisation region alone, excluding the generation and decomposition regions, as shown in Fig. 3. As a result of ozone's instability and decomposition tendencies, the lamps had to be systematically controlled, to maintain the concentration at the desired level for a prolonged period. However, it will be shown subsequently (Section 3.5), that this characteristic instability is a function of the ozone concentration.

2.3. Preparation of fabric swatches and microorganisms

6.5 cm × 6.5 cm fabric swatches with a material composition of 65% polyester and 35% cotton were utilised in this study. To ensure their sterility before commencing the experiments, they were thoroughly washed in 70% ethanol solution for up to 3 h, autoclaved and subsequently dried. Following this treatment, was exposure to a combination of ozone and UV, for a period of 40 mins.

The preparation process of the bacteria (*E. coli* and *S. aureus*) used herein, began by transferring a representative colony into 10 mL of Luria broth (Sigma Aldrich, St. Louis, USA), and incubating in a shaker at 37 °C and 150 rpm for 24 h. 1 mL of the bacteria suspension was transferred into a 1.5 mL microcentrifuge tube and centrifuged at 10,000 rpm for 5 mins. This was followed by the washing of the harvested cells with phosphate buffer saline (PBS) solution and the adjustment of the suspensions' absorbance (at 570 nm) to an optical density (OD) of 0.2 (±0.02). This corresponded to 10^9 *E. coli/S. aureus* bacteria/mL. The preparation of the fungal inoculum involved growing them on ISP2 agar plates (International *Streptomyces* Project-2 Medium) for 48 h. A 1 cm × 1 cm section of the agar piece of each fungal culture was inoculated into 100 mL of ISP2 broth to prepare seed cultures of each species by shaking them for 48 h. This was followed by an adjustment of the optical density of the two-day-old seed cultures to an optical density of 0.2 (±0.02). Required volumes of the 0.2 OD bacterial and fungal suspension were applied onto the sterilized fabric swatches aseptically, which were subsequently ozonated.

2.4. Analysis of contamination levels

Dipslides (Dip-Slides UK) were applied to ascertain the level of contamination on fabric swatches pre- and post-ozonation. For the bacterial tests, a nutrient TTC (triphenyltetrazolium chloride) agar slide was applied, whereas, fungal tests were carried out using a malt extract agar slide in this study. The slides were placed onto the desired fabric surface (pre- and post-ozonation) with gentle pressure applied for a period of 10 s, after which they were incubated at 37 °C for 24 to 72 h.

To analyse the level of microbial removal via ozonation, high-resolution images of the incubated dipslides were taken and post-processed using MATLAB's (R2020b) image processing tool, as shown
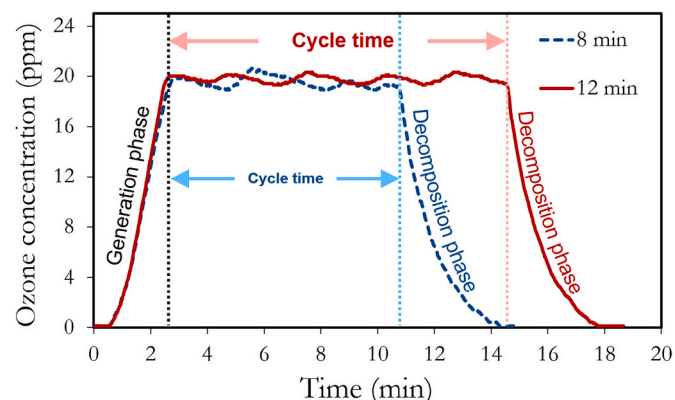

Fig. 3. A typical ozone disinfection cycle, showing the characteristic phases.

in Fig. 4. It can be observed that the tested organisms have different growth behaviours. With bacteria, distinct/separate colonies are formed, which are counted using the tool. Thus, the percentage bacterial removal can be evaluated by comparing the number of bacterial colonies before and after ozonation. The bacteria concentration per cm^2 of the dipslide agar area (5 cm × 2 cm) is calculated according to Eq. 1; where the corrected pick-up rate (CPUR) = 2 This correction factor is applied because approximately, 50% of bacteria is picked up from a surface, by the dipslide (Dipslides-UK, 2021).

$$CFUs \Big/ cm^2 = \frac{Number\ of\ colonies \times CPUR}{Agar\ area} \tag{1}$$

However, *C. albicans (CA)*, forms clustered-looking colonies, which cannot be easily counted. An evaluation of the contaminated area fraction is hence performed (Fig. 4a) to evaluate the disinfection efficiency. *A. fumigatus (AF)*, shows a totally different growth profile and was better suited for the area fraction analysis (Fig. 4b) employed for CA.

2.5. Assessment of morphological changes using Scanning Electron Microscopy (SEM)

To prepare samples for SEM, 100 μL of the bacteria suspension (Section 2.3) was added to a 10 mm by 10 mm silicon wafer and incubated in a 6-well plate at 37 °C for 4 h. The sample was then washed with PBS (0.01 M) and subsequently fixed with a solution of 2% paraformaldehyde, 2.5% glutaraldehyde and 0.1 M phosphate buffer (pH 7.4) for at least 30 min at room temperature. The fixed samples were dehydrated in gradually increasing concentrations of ethanol solutions (50, 70, 80, 90, 95 and 99% *v/v*). The specimens were subsequently treated with tert-Butanol, and then freeze-dried (Christ Alpha 1–2 LD plus) for 1 h. This was followed by gold sputter coating (Emscope SC500), after which the samples were mounted on aluminium stubs using a double-sided carbon tape and thereafter, imaged (Hitachi S-4100).

3. Results and discussion

3.1. Typical contamination levels

In a bid to achieve industrial applicability of the findings of this study, it was necessary to ascertain typical contamination levels of used garments, usually processed by large-scale cleaning and sanitisation facilities. Sample garments were obtained from Advanced Clothing Solutions (ACS) Ltd., in Motherwell, Scotland – the UK's largest clothing rental and sanitisation specialists. These garments had not undergone any cleaning process, before applying the dipslide to selected areas, which are prone to contamination (Fig. 5). As shown in Fig. 5, the armpit and groin regions of the tested garments are generally the most contaminated, except for the jacket. This is attributable to the fact that jackets are hardly in direct contact with the skin when worn.

With this data (Fig. 5), it was necessary to design the initial conditions of the experiments, to somewhat reflect this observation of bacterial concentration. Thus, a reasonable and representative microbial load can be inoculated onto the fabric swatches before ozonation is carried out.

3.2. Impact of inoculum volume and incubation duration

To ascertain the growth pattern of the organisms on the dipslide, three inoculum volumes of the test organisms were utilised (50, 100 and 200 μL) as shown in Fig. 6. The applied volumes were sufficient to saturate the entire area of the fabric swatch; this also allowed the total contaminated area of the fabric swatch to be in contact with the dipslide when pressed onto it. However, before the application of the dipslide,
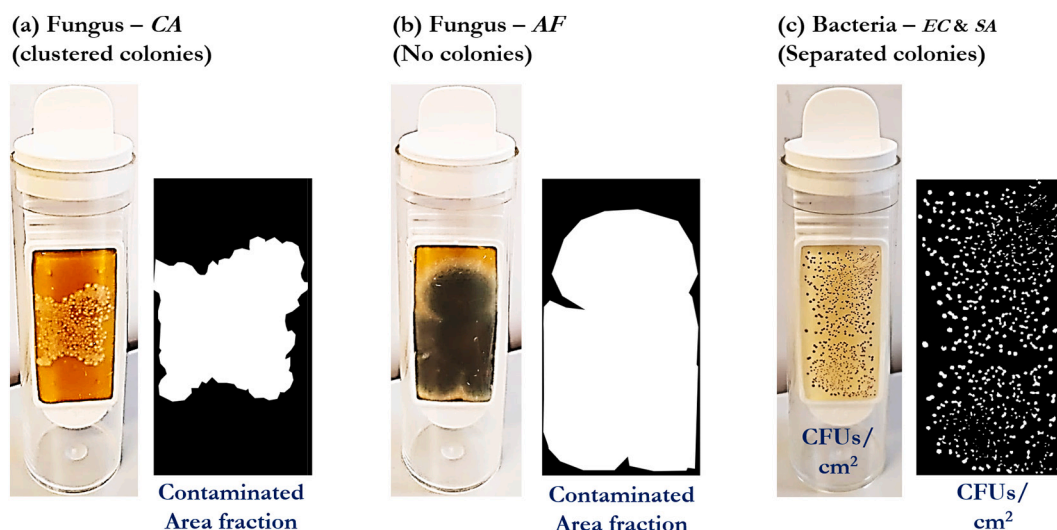
Fig. 4. Analysis of bacterial and fungal concentration via area fraction and viable counts of bacterial colonies (colony-forming units (CFU) per cm² area) of the dipslide.
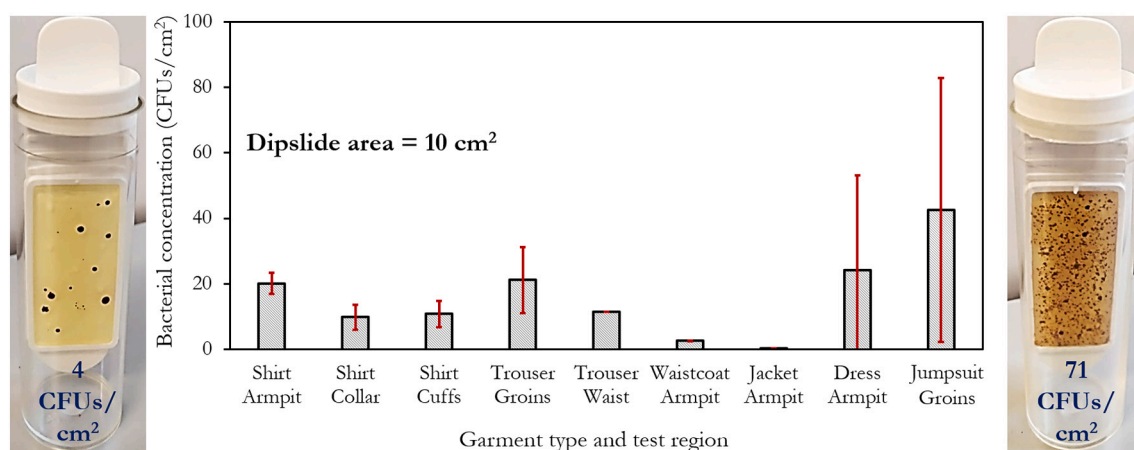

Fig. 5. Typical garment contamination levels before treatment. Data obtained from ACS Clothing, Scotland, UK. Error bars represent the standard deviation of 3 randomly selected garments of a particular type and test region. The large error bars are an indication of the inherently wide variability in the contamination levels of different garment types.

the inoculated fabric samples were left undisturbed for 15 mins to enable complete saturation of the fabric.

It can be observed that a bacteria lawn forms when 100 μL and 200 μL are applied to the fabric (Fig. 6a). This lawn area increases with the bacterial suspension volume applied (41% to 71%). Thus, 50 μL was applied to the fabric swatches when *E. coli* was tested, to allow easy and accurate comparability of the dipslides before and after ozonation. With *S. aureus*, this lawn formation was not noticed. However, 200 μL was applied to the fabric swatches (in the rest of the experiments carried out), given the relative similarity of the observed number of colonies to that of *E. coli* at 50 μL. It is also worth mentioning that these bacterial concentrations, although higher, are not farfetched from those found in used garments, as shown in Fig. 6. With both fungal suspensions, 100 μL was applied to the fabric swatches before ozonation in the chamber. Compared to *A. fumigatus*, the difference in the fungal concentration with respect to the volume of the applied suspension is more significant, with *C. albicans*.

It was also important to evaluate the dynamic growth pattern of the test organisms (particularly the fungi) at different incubation periods. Except for slight enlargement of the bacterial colonies, there were no considerable differences between 48 h and 72 h of incubation. However, it can be observed in Fig. 7, that *A. fumigatus*, showed a very rapid

change in the growth area fraction on the slide between 24 and 48 h, compared to the *C. albicans*. Thus, a 48 h incubation period of the dipslide was employed throughout this study; however, to fully ascertain 100% microbiota removal at certain test conditions, the slides were further incubated for 72 h. Sections 3.4 and 3.5 provide further ozonation conditions, which yielded complete removal of all microbiota on the slides.

3.3. Impact of ozone concentration and exposure time

The combined impacts of ozone's concentration and the duration of ozone gas treatment are shown in Fig. 8 for both *E. coli* and *S. aureus*. The generally observed trend is the increase in bacterial removal efficiency with the increase in ozonation duration, as well as ozone concentration. However, compared to *S. aureus*, in which 2 ppm was sufficient to achieve more than 99% bacterial removal, *E. coli* (gram-negative - with a thinner cell wall) proved to be more resistant to ozone at this concentration. This observation of a lower resistance of *S. aureus* to ozone was also shown in the work of Martinelli et al. (2017) when ozonated water was used for disinfection. This implies that the thickness of the microbial cell wall cannot be used as the sole measure of ozone's effectiveness against different bacteria species. An ozone concentration of 10 ppm

Fig. 6. Impact of bacterial and fungal inoculum volume on the level of growth observed on the dipslides, after 48 h of incubation. CAF: contaminated area fraction of the dipslide.

achieves a significantly better microbial load reduction of *E. coli*, compared to values obtained at 2 ppm as shown in Fig. 8a. However, complete removal of both bacteria is obtained at 20 ppm ozone concentration (Figs. 8 a and b).

The disinfection efficiencies of ozone on the tested fungi are shown in Fig. 9. As previously observed and expected, increased ozone concentration generally yields better microbial reduction. However, both *C. albicans* and *A. fumigatus* showed significantly higher resistance to ozone than the bacteria species tested. At an ozone concentration of 2 ppm, *A. fumigatus* showed the highest resistance with only a 30% reduction (at 16 mins) compared to the other organisms (54% for *C. albicans*, 94% for *E. coli* and 99.9% for *S. aureus*). This finding has also been substantiated by Hudson and Sharma (2009), where one treatment cycle was sufficient to remove *C. albicans* compared to two treatment cycles required for *A. fumigatus*. As with the tested bacteria (Fig. 8), 20

ppm and 4 mins exposure were sufficient to yield satisfactory inhibition of fungal growth. To ascertain the completeness of *A. fumigatus* removal from the swatch by ozone, the dipslides were further incubated for 24 h (72 h in total), and no growth was observed on all dispslides after ozone treatments at 20 ppm.

As part of the efforts to validate the findings of this work on industrial systems, we have also tested the efficacy of ozone at 2 ppm on full garments (4 shirts), in ACS's ozone facility. The 4 shirts used, were inoculated with 50, 200, 100 and 100 μL of *E. coli*, *S. aureus*, *C. albicans* and *A. fumigatus*, suspensions, respectively (same suspensions as those used in the lab). This was performed at designated locations on the garment for easy identification of the inoculated region after ozonation. These garments were placed in the industrial ozonation chamber (13.4 m × 2.5 m × 2.7 m) originally containing up to 500 garments. The results shown in Fig. 10, illustrate satisfactory similarity between the
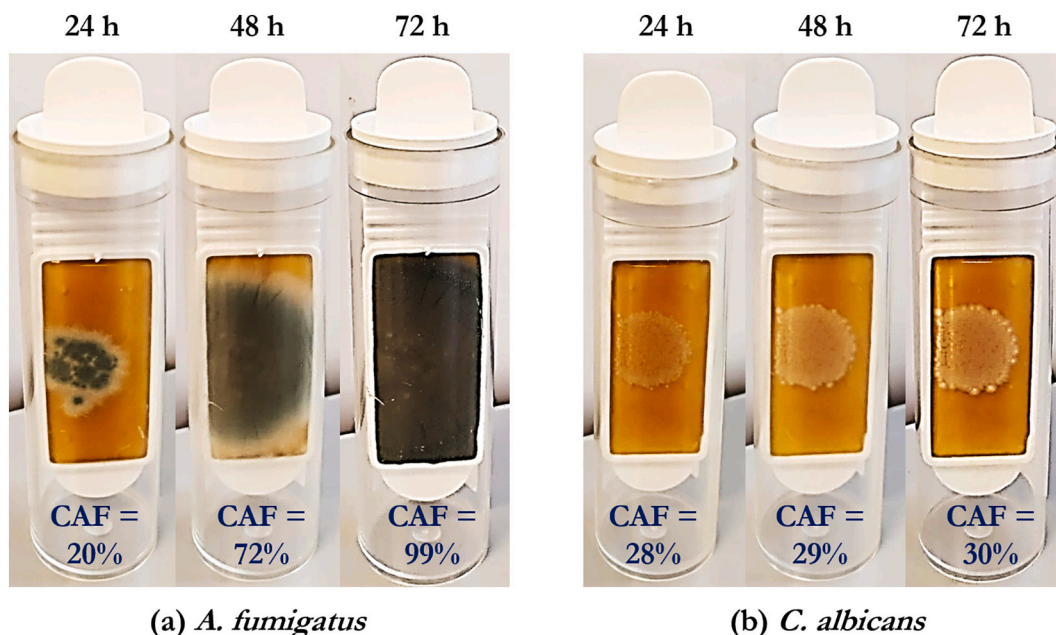
24 h **48 h** **72 h**   **24 h** **48 h** **72 h**

(a) A. fumigatus   **(b) C. albicans**

Fig. 7. Fungal growth on dipslide after 24 h, 48 h and 72 h of incubation at 37 °C. The dipslide was applied to a fabric swatch inoculated with 50 μL of the tested organism. CAF: contaminated area fraction of the dipslide.
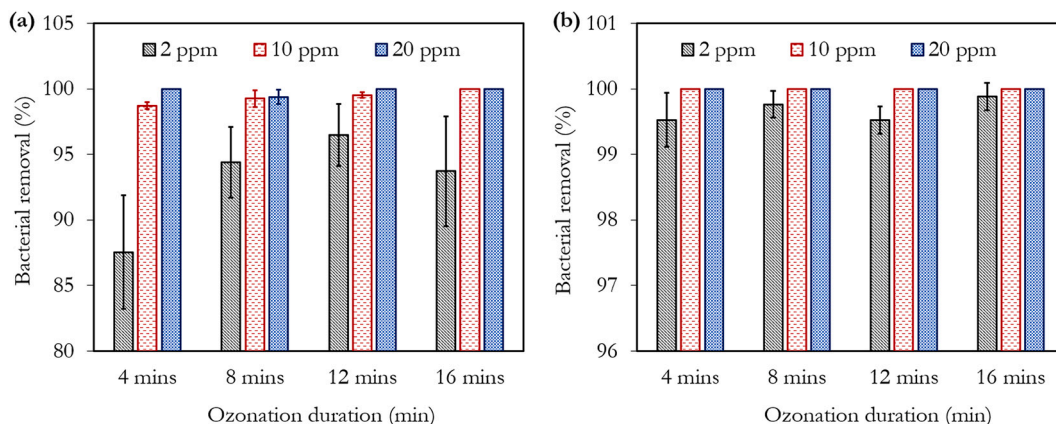

Fig. 8. Effect of ozone concentration and exposure time on the bacterial removal efficiency (a) *E. coli* (b) *S. aureus*, after an incubation period of 48 h. Error bars represent the standard deviation of 3 runs.
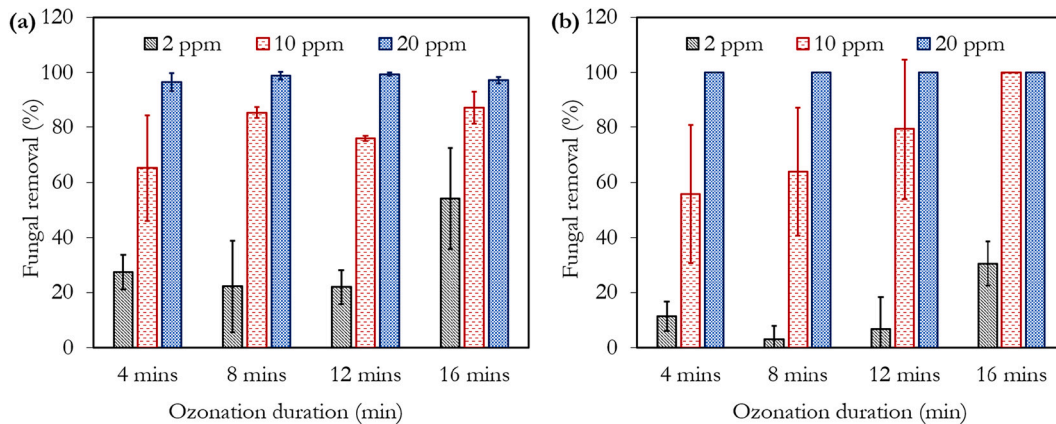

Fig. 9. Effect of ozone concentration and exposure time on the fungal removal efficiency (a) *C. albicans* (b) *A. fumigatus*, after an incubation period of 48 h. Error bars represent the standard deviation of 3 runs.
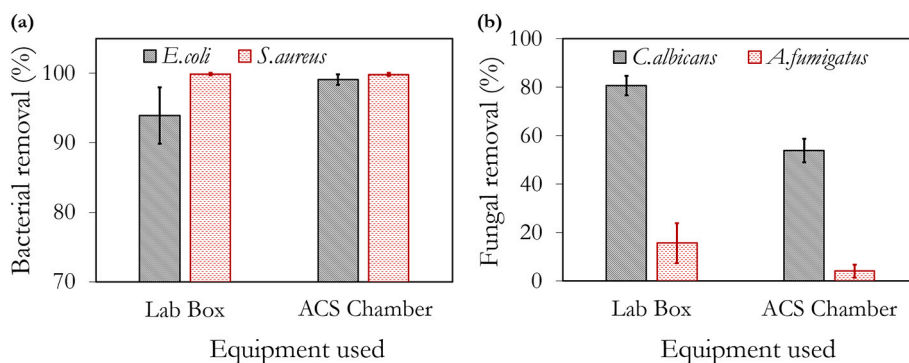
Fig. 10. Result validation against industrial ozonation system (2 ppm for 20 mins). Error bars represent the standard deviation of 3 runs.

results obtained using ACS's chamber and the lab-based chamber for bacterial inactivation. The increased number of garments in ACS's chamber, which may act as obstacles to efficient gas penetration into the test garments did not adversely affect the bacterial disinfection efficiency. This can be attributed to the strong gas agitation maintained in the chamber; this sort of agitation is only beneficial when all areas of the garment are exposed to ozone. However, a more significant difference between the fungal removal efficiencies of the small-scale chamber and the larger chamber is observed (Fig. 10b). This may have been due to differences in the ambient temperatures, of the lab-based box (which was in a more controlled environment – fume cupboard) and the larger chamber (which is situated in an open-air environment); thus potentially affecting ozone's stability in the large chamber.

Preliminary tests on our lab-based sterilisation chamber (Fig. 1), have also shown considerable differences between hanging the fabric swatch (via the rotatable anchor) versus placing it on a petri dish in the chamber. The latter reduces the exposure area of the swatch to ozone significantly, despite the application of gas agitation. With *S. aureus*, the bacterial reduction efficiency increased from 60% to 99% by changing the swatch's position from flat (on a petri dish) to hanging on the anchor.

3.4. Analysis of bacterial inactivation via scanning electron microscopy (SEM)

Visualisations via SEM micrographs provide information on possible mechanisms governing the reduction in the growth of the organism (*E. coli*), as previously presented. Fig. 11b shows the effect of ozone exposure at 20 ppm for 8 mins. As can be observed, the main morphological change created by ozone gas is the disruption and deformation of
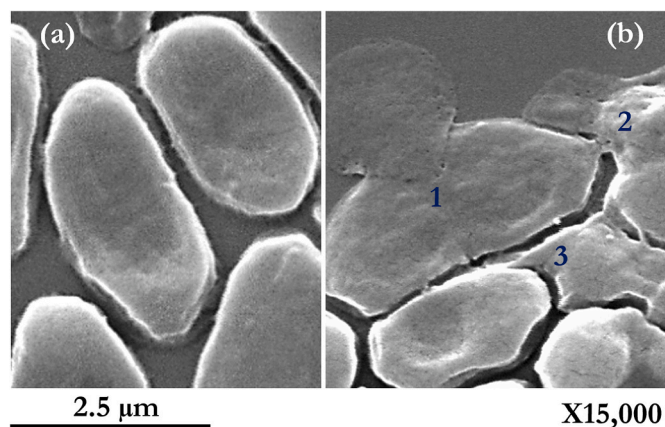
the cell wall (resulting in bacterial leakage as shown in regions 1 and a roughened structure as shown in regions 2 and 3), compared to the regular smooth-shaped structured cells seen in the non-treated scenario (Fig. 11a). This disruption ultimately affects the cells' viability, thus leading to the eventual inactivation. This mechanism portrayed by our SEM micrographs is also similar to those reported in Patil et al. (2011) and dos Santos et al. (2021).

3.5. Design implications for rapid ozone disinfection systems

Most disinfections studies on the use of ozone in literature, have either reported relatively long cycle times required for microbial inactivation or too high concentrations (up to 1500 ppm - Kowalski et al., 1998, 2003), which are difficult to reach in a timely manner in large-scale applications. Yano et al. (2020) demonstrated the efficacy of gaseous ozone treatment on the novel coronavirus (SARS-CoV-2). It up took 60 min of exposure at 1 ppm to yield up to 90% inactivation of the virus. Thus, there is a growing need for even more rapid, effective, scalable and safe sanitisation systems particularly in this era of the Covid-19 pandemic. While high concentrations may help reduce the microbial inactivation time, very high concentrations may have a reverse effect of prolonging the other phases of the total cycle time (generation and decomposition). Furthermore, garment exposure at very high concentrations for prolonged periods may adversely affect the mechanical properties of the fibres in textile processing. Wakida et al. (2004) reported that silk fabric may become crisper after ozone gas treatment at very high concentrations; whereas, Ben Fraj and Jaouachi (2021) reported up to 15% shrinkage, during ozone treatment of denim fabric in water at a concentration of 55 g/N.m^3.

Furthermore, very high concentrations become difficult to manage in industrial settings, especially in the interest of worker safety, and compliance with COSHH regulations on the worker exposure limit. Ozone's natural instability, advances in catalyst development tailored to ozone gas and appropriately sized mechanical extraction systems for purging ozone gas from the chamber, are key factors that determine the efficiency of ozone's decomposition. Fig. 12 demonstrates the need for an adequate selection of gas suction/removal equipment. It can be observed that once a critical extraction rate of 28 m^3/h is reached in our lab-based sterilisation chamber (0.21 m^3), there is no further improvement in the decomposition rate of ozone. Furthermore, the instability of ozone tends to be more significant at higher concentrations (20 ppm), than at a lower concentration (10 ppm – Fig. 12); both curves were obtained at 20 °C and 50% relative humidity. This is specifically observed in the high concentration regions of the curves. While the 10 ppm curve appears relatively flat for the 2-min duration, the 20 ppm curve, showed a gently downward sloping behaviour. This may be attributed to a high recombination rate of the UV-generated free oxygen radicals to form oxygen molecules at higher concentrations compared to low ozone concentrations. Adequate sizing and selection of mechanical extraction equipment are also dependent on the peak ozone

Fig. 11. Scanning electron microscopy of *E. coli* nontreated (a) and ozone-treated (b). Ozone treatment was carried out at 20 ppm for 8 mins. Region 1 shows leakage of material from the bacteria; regions 2 and 3 show deformation of the cell and disruption of the cell membrane.
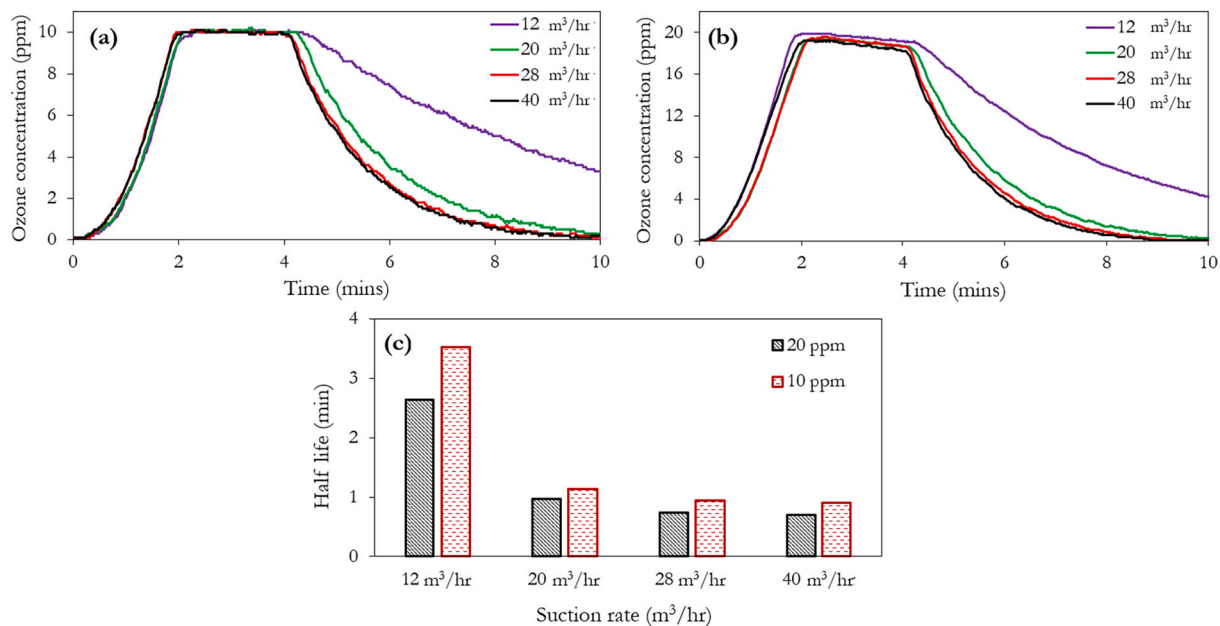
Fig. 12. Impact of suction flow rate on decomposition profile and half-life. UV lamps are switched off once the desired concentration is reached (10 ppm and 20 ppm, respectively); the gas is allowed to circulate in the chamber for 2 min after which the extraction commences.

concentrations attainable in the test chamber.

4. Conclusions

A concentration-time analysis of ozone disinfection capabilities on common environmental and pathogenic bacteria and fungi using garment substrates has been performed. The presented results show that the resistance of the tested organisms to ozone gas is in the order *A. fumigatus* > *C. albicans* > *E. coli* > *S. aureus*. The sufficient ozone concentration and exposure time required to yield complete microbial removal are 20 ppm and 4 mins, respectively. The presented results of this work further demonstrate the necessity of maintaining an informed trade-off between ozone concentration and exposure duration. While large-scale garment processing industries like ACS Clothing will benefit from ozone systems capable of rapidly disinfecting garments, the impact of ozone on the garment fibre properties, and the need to install efficient ozone destruction systems for safe operations, are important factors to consider.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

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