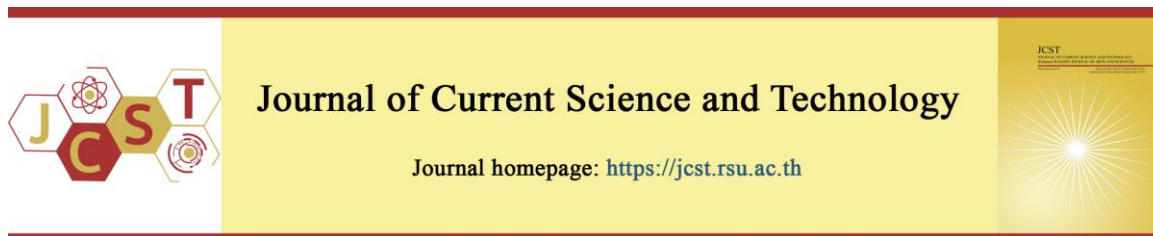


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Microbial Ozone Decontamination of N95 Respirators: Efficacy and Material Preservation

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Abstract

Ozone gas is a promising method for decontaminating personal protective equipment (PPE), providing broad antimicrobial activity with minimal residue effects. However, its effects on the structural integrity and filtration performance of N95 respirators are not well established. This study evaluated the antimicrobial efficacy of ozone treatment against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans* on culture media and N95 respirators, and assessed whether fiber integrity and filtration efficiency were preserved using the GermZero3 prototype sterilizer developed with the National Science and Technology Development Agency (NSTDA). Microbial suspensions (10^4 CFU/mL in TSB broth) were inoculated onto agar plates and respirator sections, and then exposed to 25–50 ppm ozone for 15–60 min. Viability was assessed by culture, while fiber integrity and filtration efficiency were evaluated by scanning electron microscopy and a NaCl aerosol test. Complete eradication of *P. aeruginosa* was achieved after 15 min and *S. aureus* within 45 min. *C. albicans* showed 99.90–99.98% reduction by 45–60 min, with no statistically significant difference from full clearance. When applied to contaminated respirators, ozone treatment eliminated all three pathogens after 60 min. Fiber morphology remained intact, and filtration efficiency was preserved at 99.99%, exceeding the $\geq 95\%$ N95 standard. These findings support ozone treatment with the GermZero3 sterilizer as a safe and effective method for extending N95 respirator use during shortages.

Keywords: germzero3 prototype; ozone decontamination; n95 respirators

1. Introduction

The COVID-19 pandemic has highlighted the critical importance of personal protective equipment (PPE) in safeguarding healthcare workers against infectious agents. Among various PPE, N95 respirators have been essential for providing respiratory protection, particularly in high-risk clinical environments. While their role in protecting against viruses like SARS-CoV-2 is well-established, N95 respirators are also critical for preventing the inhalation of a wide range of other infectious agents,

including airborne bacterial and fungal spores. However, global PPE shortages during pandemic surges have posed significant challenges to healthcare systems, including pediatric healthcare facilities, where vulnerable patient populations require stringent infection control measures (Del Monte et al., 2024; Jain, 2020; Kharbat et al., 2020).

To address these shortages, several decontamination methods such as ultraviolet-C (UV-C) irradiation, moist heat, and vaporized hydrogen peroxide (Biabani et al., 2025; Hanyanunt et al., 2020; Kharbat et al., 2020;

Kumar et al., 2021) have been explored. Despite their potential, concerns remain regarding material degradation, inconsistent microbial inactivation, and limited scalability (Biabani et al., 2025; Choudhury et al., 2024; Hanyanunt et al., 2020; Kharbat et al., 2020; Kumar et al., 2021). In this context, ozone gas has emerged as a promising alternative (Tippayawat et al., 2022). Ozone offers broad-spectrum antimicrobial activity and effective penetration of porous materials such as multilayered layers, and minimal toxic residue when adequately aerated (Choudhury et al., 2024; Kharbat et al., 2020; Manning et al., 2021; Tippayawat et al., 2022).

Several previous studies have demonstrated ozone's potent virucidal, bactericidal, and fungicidal effects (Cristiano, 2020; Epelle et al., 2022; Manning et al., 2021; Rangel et al., 2022; Sallustio et al., 2021; Sharma & Hudson, 2008). It has been shown to effectively inactivate both enveloped and non-enveloped viruses, including SARS-CoV-2 (Cristiano, 2020; Sallustio et al., 2021), and to eliminate a wide range of bacteria such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococcus faecalis*, and *Streptococcus mutans* as well as fungi like *Candida albicans* and *Aspergillus fumigatus* (Santos et al., 2021; Epelle et al., 2022; Epelle et al., 2023a; Panebianco et al., 2022; Rangel et al., 2022; Sharma & Hudson, 2008). The primary mechanism involves oxidative stress that damages microbial membranes, proteins, and nucleic acids, ultimately leading to irreversible cell death (Santos et al., 2021; Epelle et al., 2022; Rangel et al., 2022; Sharma & Hudson, 2008).

Nevertheless, few studies have systematically evaluated the effects of ozone on N95 respirator material properties, particularly fiber morphology and standardized particle filtration efficiency (PFE). Most prior reports emphasized microbial inactivation alone, often using high ozone concentrations or extended exposure times that may not be practical in clinical settings. Moreover, evidence regarding the application of newly developed prototype ozone sterilizers remains scarce. Therefore, this study aimed to evaluate the antimicrobial efficacy of ozone treatment against representative bacterial and fungal pathogens (*P. aeruginosa*, *S. aureus*, and *C. albicans*) on both culture media and N95 respirators, while also assessing mask fiber integrity and PFE preservation using the GermZero3 prototype sterilizer, which was developed with the National Science and Technology Development Agency (NSTDA).

2. Objectives

This study aimed to evaluate the antimicrobial efficacy of ozone treatment against *P. aeruginosa*, *S. aureus*, and *C. albicans* on both culture media and N95 respirators using the GermZero3 prototype ozone sterilizer. In addition, the study aimed to assess the impact of ozone exposure on the fiber integrity and particle filtration efficiency of N95 respirators.

3. Materials and Methods

3.1 Ozone Generator System

Ozone treatment was conducted using the GermZero3 prototype ozone sterilizer, a custom-built unit developed in collaboration with the National Science and Technology Development Agency (NSTDA), Thailand. The device (220V, 116W) integrates an ozone generation system and an automatic gas neutralization module within a sealed chamber to ensure effective microbial inactivation and operational safety. The system was capable of generating ozone concentrations between 25 and 50 parts per million (ppm), as validated by internal calibration. An internal circulation mechanism facilitated uniform ozone distribution and prevented heat accumulation during operation. The device also allowed setting exposure durations between 15 and 60 min, depending on experimental requirements. Following each disinfection cycle, an automatic ozone neutralization phase was activated via activated carbon filtration, ensuring residual ozone was reduced to safe levels prior to chamber access. This closed-loop configuration minimized environmental ozone exposure and supported safe handling protocols.

3.2 Evaluation of Ozone Generator Efficiency in Microbial Decontamination

To evaluate the efficiency of ozone treatment in microbial decontamination, three standard strains of representative common pathogenic microorganisms (*P. aeruginosa* ATCC 27853, *S. aureus* ATCC 25523, and *C. albicans* ATCC 10231) were used. The strains were stored at -20°C until use and cultured in Tryptic Soy Broth (TSB; Difco™, Becton, Dickinson and Company, Sparks, MD, USA) at 37°C for 24 h. Microbial suspensions were adjusted with TSB to a final concentration of 10^4 CFU/mL, corresponding to a turbidity of 0.25 McFarland standard following a previous study (Santos et al., 2021). A 50 μL of each suspension was evenly spread onto Tryptic Soy Agar (TSA; Difco™, Becton, Dickinson and Company, Sparks, MD, USA) for *P. aeruginosa* and *S. aureus*, and Sabouraud Dextrose Agar (SDA; Difco™, Becton,

Dickinson and Company, Sparks, MD, USA) for *C. albicans*. The inoculated plates were then exposed to ozone treatment for 15, 30, 45, and 60 min. Microbial viability was determined by counting colony-forming units (CFU) after incubation at 37 °C for 24 h. Positive and negative controls were included, and all experiments were performed in triplicate. The proportion of microbial clearance (%) was calculated using the formula: $[(\text{initial CFU} - \text{surviving CFU}) / \text{initial CFU}] \times 100$, based on three independent replicates for each organism and time point. Combined clearance values were calculated from the total CFU counts of *P. aeruginosa*, *S. aureus*, and *C. albicans* at each time point, and the overall clearance percentage was determined relative to the pooled baseline inoculum.

Statistical analyses were conducted using STATA/BE Software, Version 18.0 (Stata Corp, College Station, TX, USA). Laboratory results were described as proportions representing microbial clearance. To evaluate the effectiveness of ozone exposure in eliminating microbial viability at independent time intervals (15, 30, 45, and 60 min), proportions were compared. The null hypothesis assumed 100% microbial clearance at each time point. Accordingly, one-sample test of proportion was used to compare observed clearance rates against the 100% benchmark. All analyses were performed at a 95%

confidence level, with *p-values* <0.05 considered statistically significant.

3.3 Ozone Treatment of N95 Respirators: Evaluation of Microbial Decontamination

To evaluate the efficacy of ozone treatment on N95 respirators, sections of commercially available N95 masks (3M, St. Paul, MN, USA) were cut into 1 × 1 cm pieces. Prior to use, each section was sterilized with ethylene oxide (EtO) following standard hospital sterilization protocols to ensure complete decontamination (Rutala & Weber, 2004). Standard strains prepared as described above were inoculated onto the surface of individual N95 sections. The contaminated mask pieces were then subjected to ozone exposure for 60 min.

Following ozone treatment, each N95 section was transferred into 5 mL of TSB and incubated at 37 °C to allow enrichment of surviving microorganisms. A 50-μL aliquot of the broth culture was subsequently spread onto Tryptic Soy Agar (TSA; Difco™, Becton, Dickinson and Company, Sparks, MD, USA) for detection of *P. aeruginosa* and *S. aureus*, and onto Sabouraud Dextrose Agar (SDA; Difco™, Becton, Dickinson and Company, Sparks, MD, USA) for detection of *C. albicans*. The inoculated plates were incubated at 37 °C and examined for microbial growth at 24 and 48 h. All experiments were performed in triplicate.

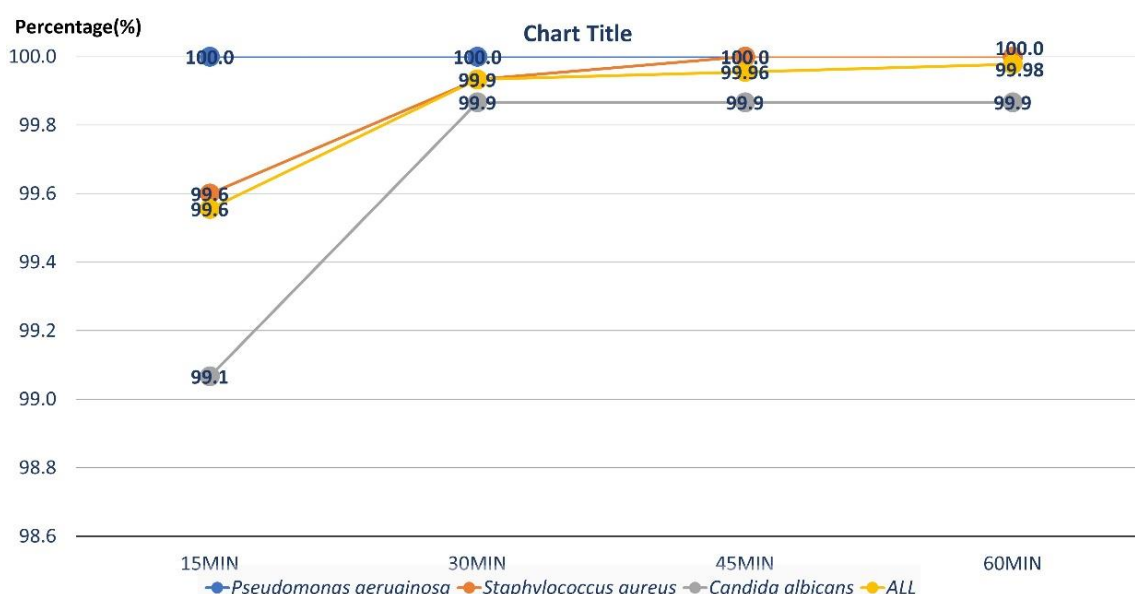


Figure 1 Percentage reduction in CFU of *P. aeruginosa*, *S. aureus*, and *C. albicans* after ozone exposure for 15, 30, 45, and 60 min. The combined clearance rate across all organisms reached ≥99.96% by 45 min.

3.4 Fiber Integrity of N95 Respirators

In addition to microbial decontamination, fiber morphology of the N95 respirator filter layer was examined via scanning electron microscopy at King Mongkut's University of Technology Thonburi (KMUTT), Thailand. Samples of mask section (5×5 mm) were sputter-coated with a conductive metal and imaged at multiple magnifications. For each respirator, three replicate mask sections were analyzed, with imaging performed on three regions per section (left, middle, right).

3.5 Filtration Efficiency of N95 Respirators

Particle filtration efficiency (PFE) was evaluated using a NaCl aerosol challenge test according to the Thai Industrial Standard (TIS 62048-2562; flow rate 85 L/min). Mask samples were submitted to the Regional University Environmental Engineering (RUEE) Center, Lanna, Thailand, for testing. Two unused respirators (controls; n = 2) and three respirators subjected to ozone exposure for 60 min (treated; n = 3) were sealed in sterile containers and transported under ambient conditions. At RUEE, each respirator was tested individually, and the results were reported as PFE (%) for each article, with group averages calculated accordingly.

4. Results

4.1 Evaluation of Ozone Generator Efficiency in Microbial Decontamination

To evaluate the antimicrobial efficacy of ozone treatment, microbial clearance was assessed at 15, 30, 45, and 60 min against three representative organisms (*P. aeruginosa*, *S. aureus*, and *C. albicans*). Overall clearance reached 99.96% at 45 min and 99.98% at 60 min, with no statistically significant difference from complete elimination ($p = 0.179$ and $p = 0.343$) (Table 1). As shown in Figure 1, ozone exposure produced substantial time-dependent reductions across all organisms, with the combined clearance rate reaching $\geq 99.96\%$ by 45 min. *P. aeruginosa* demonstrated the highest susceptibility, achieving complete clearance

after 15 min and maintaining eradication at all subsequent time points. *S. aureus* showed progressive reduction, achieving full clearance by 45 min. In contrast, *C. albicans* exhibited slower reduction, reaching 99.90% at 45 min and 60 min. Although residual colonies were occasionally observed, the values were statistically indistinguishable from complete elimination.

4.2 Ozone Treatment of N95 Respirators: Evaluation of Microbial Decontamination

Ozone exposure of N95 respirators inoculated with *P. aeruginosa*, *S. aureus*, and *C. albicans* for 60 min resulted in complete microbial elimination. No microbial growth was detected after enrichment in TSB and subsequent plating on agar, indicating complete elimination of viable organisms (Table 2). The 60-minute exposure time was selected based on preliminary plate-based experiments in which clearance rates consistently reached $\geq 99.96\%$ for all tested organisms and showed no statistically significant difference from complete elimination ($p > 0.05$). These findings confirm that a 60-minute ozone treatment provides reliable and effective disinfection suitable for N95 respirator application.

4.3 Fiber Integrity of N95 Respirators

Scanning electron microscopy (SEM) was performed to assess the impact of ozone exposure on the fiber morphology of N95 respirators. Untreated control masks and ozone-treated masks (60 min) were examined at multiple magnifications. For each respirator, three replicate sections were analyzed, with imaging performed in three regions per section (left, middle, right). SEM analysis showed that the fibers of ozone-treated masks retained their original morphology, with no evidence of thinning, breakage, fusion, or surface irregularities compared with untreated controls (Figure 2). These findings indicate that a 60-minute ozone treatment does not compromise the structural integrity of N95 mask fibers.

Table 1 Proportion of microbial clearance (%) for *P. aeruginosa*, *S. aureus*, and *C. albicans* after ozone exposure at 15, 30, 45, and 60 min, with combined values. P-values represent comparisons with the hypothesized complete elimination (100%).

Organism	Proportion of culture				P-value			
	15min	30min	45min	60min	15min	30min	45min	60min
<i>Pseudomonas aeruginosa</i>	100.00	100.00	100.00	100.00	1.000	1.000	1.000	1.000
<i>Staphylococcus aureus</i>	99.60	99.90	100.00	100.00	0.014*	0.221	1.000	1.000
<i>Candida albicans</i>	99.10	99.90	99.90	99.90	<0.001*	0.221	0.221	0.221
Combined value	99.60	99.90	99.96	99.98	<0.001*	0.034*	0.179	0.343

Note: *Indicates statistical significance at $p < 0.05$.

Table 2 Microbial recovery from N95 respirators after ozone treatment

Organism	Condition	Enrichment (TSB)	Plating (TSA/SDA)	Result
<i>P. aeruginosa</i>	Positive control (inoculated, no ozone)	Turbid	Colonies present	Growth
	Ozone-treated (60 min)	Clear	No colonies	Eliminated
	Negative control (uninoculated)	Clear	No colonies	No growth
<i>S. aureus</i>	Positive control	Turbid	Colonies present	Growth
	Ozone-treated	Clear	No colonies	Eliminated
	Negative control	Clear	No colonies	No growth
<i>C. albicans</i>	Positive control	Turbid	Colonies present	Growth
	Ozone-treated	Clear	No colonies	Eliminated
	Negative control	Clear	No colonies	No growth

Note: Positive control = inoculated masks without ozone; Ozone-treated = inoculated masks exposed to ozone for 60 min; Negative control = uninoculated masks. Growth was determined by turbidity in enrichment broth (TSB) and colony formation on TSA (*P. aeruginosa*, *S. aureus*) or SDA (*C. albicans*).

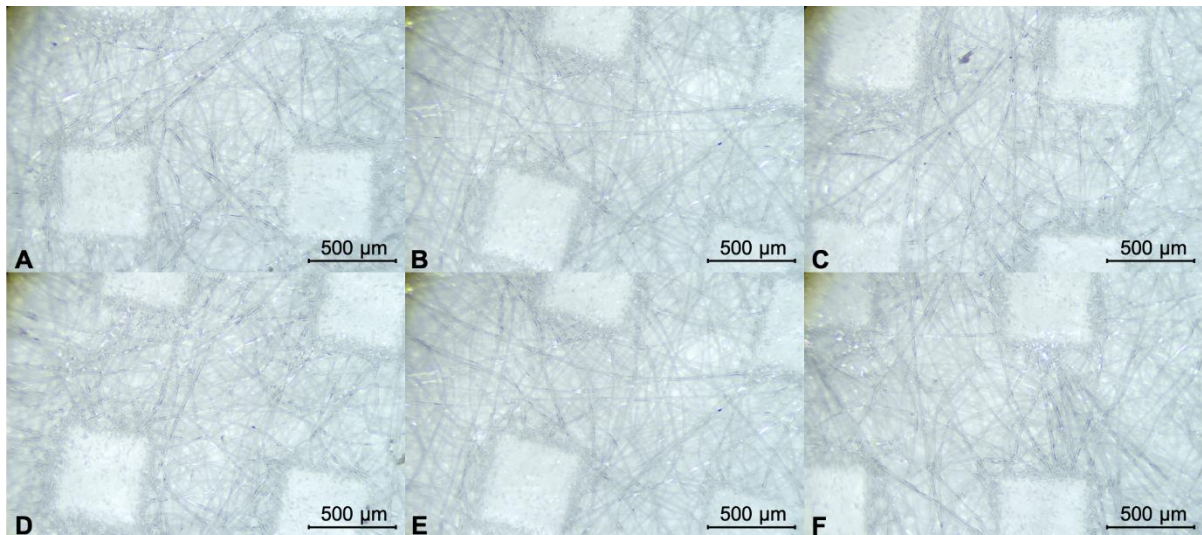


Figure 2 Scanning electron microscopy (SEM) images of N95 respirator fibers with and without ozone treatment. (A–C) Untreated control masks: left, middle, and right regions. (D–F) Ozone-treated masks (60 min exposure): left, middle, and right regions.

4.4 Filtration Efficiency of N95 Respirators

The particle filtration efficiency (PFE) of N95 respirators was evaluated in unused control masks (n=2; Test Article Numbers 1–2) and ozone-treated masks (n=3; Test Article Numbers 3–5) using a NaCl aerosol challenge test according to the Thai Industrial Standard (TIS 62048-2562; flow rate 85 L/min). All samples demonstrated filtration efficiencies of 99.99%, well above the $\geq 95\%$ threshold required for N95 certification. No difference was observed between control and ozone-treated groups, confirming that a 60-minute ozone exposure did not compromise respirator filtration performance (Table 3).

Table 3 Particle filtration efficiency (PFE) of unused control and ozone-treated N95 respirators

Group	Test Article Number	PFE (%)
Control (unused)	1	99.99
	2	99.99
	3	99.99
Treated (ozone)	4	99.99
	5	99.99

5. Discussion

The reuse of N95 respirators has been a critical issue during pandemic-related shortages, particularly in high-risk healthcare settings (Del Monte et al., 2024; Jain, 2020). Multiple decontamination strategies have been investigated, including ultraviolet-C irradiation, moist heat, and vaporized hydrogen peroxide, but

challenges remain, particularly material degradation, inconsistency of microbial inactivation, and limited scalability (Biabani et al., 2025; Choudhury et al., 2024; Epelle et al., 2022; Kumar et al., 2021; Tipayawat et al., 2022). Ozone gas has gained attention due to its strong oxidative potential, capacity for deep penetration into multilayered respirator materials, and minimal residual toxicity when adequately neutralized (Santos et al., 2021; Epelle et al., 2022; Rangel et al., 2022).

The selection of *P. aeruginosa*, *S. aureus*, and *C. albicans* in this study was intentional to represent a broad microbial spectrum Gram-negative bacteria, Gram-positive bacteria, and fungi covering key pathogens relevant to hospital and respiratory tract infections (Duan et al., 2020; Hurley, 2025). Although previous studies have demonstrated the virucidal potential of ozone against respiratory viruses, including SARS-CoV-2 (Choudhury et al., 2024; Cristiano, 2020; Lam & Phil, 2023; Kumar et al., 2021; Sallustio et al., 2021), this study was limited to culturable organisms due to biosafety and laboratory infrastructure constraints. Nevertheless, the chosen organisms span a practical resistance gradient, with fungi representing the most ozone-resistant and Gram-negative bacteria the most susceptible, consistent with prior findings.

In this study, ozone treatment using the GermZero3 prototype sterilizer at 25–50 ppm demonstrated broad-spectrum antimicrobial efficacy. *P. aeruginosa* was the most susceptible, being eradicated within 15 min, whereas *S. aureus* required 45 min for complete clearance. *C. albicans* exhibited greater resistance, requiring 60 min for elimination. These results align with prior work indicating variable microbial susceptibilities to ozone, which reflect structural differences in microorganisms. Gram-negative bacteria including *P. aeruginosa* possess a thinner peptidoglycan layer and a more permeable outer membrane, making them more vulnerable to oxidative disruption. In contrast, Gram-positive bacteria like *S. aureus* have a much thicker peptidoglycan layer that provides structural protection, resulting in slower inactivation. Yeasts including *C. albicans* display the highest tolerance, attributable to their complex cell wall containing chitin, glucans, and mannoproteins, along with an ergosterol-rich membrane. These features confer greater resistance to oxidative stress and help explain the longer exposure times required for clearance (Santos et al., 2021; Epelle et al., 2022; Moore et al., 2000; Panebianco et al., 2022; Rangel et al., 2022; Sharma & Hudson, 2008). Importantly, when applied to N95 respirators artificially contaminated with these pathogens, a 60-minute exposure achieved

complete microbial elimination without detectable survivors in enrichment or plating assays (Santos et al., 2021).

Parallel analyses confirmed that respirator fibers remained intact following ozone exposure, with no evidence of thinning, breakage, or fusion on SEM imaging. Furthermore, NaCl aerosol challenge testing demonstrated that particle filtration efficiency was preserved at 99.99% in both treated and untreated respirators, exceeding the $\geq 95\%$ threshold for N95 certification. These findings indicate that ozone not only achieves effective disinfection but also maintains respirator integrity and protective function.

Previous studies have evaluated ozone decontamination of N95 respirators, focusing primarily on viral inactivation, particularly SARS-CoV-2, or on limited bacterial species, with some reporting preserved filtration performance after treatment (Manning et al., 2020; Sharma et al., 2022). However, most did not simultaneously address a broad microbial spectrum or comprehensively assess respirator material properties. This study extends prior work by incorporating Gram-negative, Gram-positive, and fungal pathogens, while also examining fiber morphology and standardized particle filtration efficiency, thereby providing a more integrated evaluation of ozone as a practical decontamination strategy for N95 reuse.

Compared with other reprocessing methods, ozone is a powerful antimicrobial agent with broad-spectrum efficacy and superior penetration into multilayered respirator media, unlike UV-C, which acts mainly on exposed surfaces. Unlike vaporized hydrogen peroxide, ozone diffuses effectively across filter layers without leaving harmful residues and decomposes into oxygen, making it environmentally friendly and safer than ethylene oxide (EtO). It offers operational flexibility in gaseous, aqueous, or mist forms and is suitable for heat-sensitive materials. Importantly, repeated ozone exposures did not compromise N95 filtration efficiency (Epelle et al., 2023b; Manning et al., 2021; Sharma et al., 2022; Zhu et al., 2022). Nonetheless, several limitations should be acknowledged. This study evaluated only a single respirator model, did not investigate the effects of repeated ozone exposure cycles, and did not directly assess viral pathogens. In addition, spore-forming fungi such as *Aspergillus* spp., which may be more resistant to ozone, were not evaluated. Moreover, respirator sections were inoculated with standardized microbial suspensions, which do not fully replicate real-world contamination involving aerosolized

droplets, repeated use, and organic materials. Future research should expand to different respirator models, multiple decontamination cycles, direct viral inactivation studies, and testing against spore-forming fungi, as well as incorporating more realistic contamination models and assess respirator fit and durability under real-world conditions, to establish the scalability of ozone-based sterilization in healthcare practice.

6. Conclusion

Ozone treatment using the GermZero3 prototype sterilizer at 25–50 ppm for 60 min effectively eliminated representative bacterial and fungal pathogens from N95 respirators while preserving fiber morphology and maintaining particle filtration efficiency at 99.99%, well above the $\geq 95\%$ N95 standard. These findings demonstrate that ozone is a safe, practical, and residue-free decontamination strategy to enable N95 respirator reuse during critical supply shortages. Further validation across respirator models, repeated decontamination cycles, and clinical performance assessments is warranted to support large-scale implementation in healthcare settings.

7. Abbreviations

Abbreviation	Full Term
CFU	Colony-Forming Unit
EtO	Ethylene Oxide
PFE	Particle Filtration Efficiency
PPE	Personal Protective Equipment
ppm	Parts Per Million
SDA	Sabouraud Dextrose Agar
SEM	Scanning Electron Microscopy
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth

8. Credit Statement

Tanit Boonsiri: Conceptualization, Methodology, Investigation, Data Curation, Writing – Original Draft, Funding Acquisition.

Pimwan Thongdee: Investigation, Validation, Laboratory Experiments, Data Curation.

Sirachat Nitchaphanit: Investigation, Laboratory Experiments, Data Curation.

Nitchatorn Sungsirin: Resources, Laboratory Experiments, Data Curation.

Piyanate Kesakomol: Investigation, Laboratory Experiments, Data Curation.

Sethapong Lertsakulbunlue: Statistical Analysis, Formal Analysis, Writing – Review & Editing.

Phoempon Siangdang: Laboratory Experiments, Resources, Validation, Methodology.

Yeampon Nakaramontri: Laboratory Experiments, Resources, Validation, Methodology.

Veerachai Watanaveeradej: Supervision, Writing – Review & Editing, Project Administration.

Passara Wongthai: Conceptualization, Methodology, Supervision, Writing – Original Draft, Writing – Review & Editing, Project Administration.

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