Centrum Rzeczoznawstwa Budowlanego

Inżynieria Bezpieczeństwa Obiektów Antropogenicznych 4 (2024) 71-80
https://inzynieriabezpieczenstwa.com.pl/

Inżynieria Bezpieczeństwa Obiektów Antropogenicznych

Selected methods for effective inactivation of microorganisms in experimental chambers intended for indoor air bioaerosol studies

Rafał B. LEWANDOWSKI [1*], Zygmunt MIERCZYK [2]

[1] *Biomedical Engineering Centre, Institute of Optoelectronics, Military University of Technology, Warsaw, Poland*

Abstract

The frequent occurrence of threats from various airborne biological factors has led to a significant increase in experimental research on bioaerosols in recent years. One of the key areas of interest for researchers in this field are microorganisms present in enclosed spaces, both suspended in indoor air and settled on surfaces. A common feature of all these experiments in the initial stage of the investigation is the simulation of basic phenomena related to bioaerosols based on closed experimental systems. During this type of research, a crucial preliminary step that significantly impacts the quality of biological research results is the process of ensuring the appropriate microbiological purity of the experimental cells. The filtration methods currently used for indoor air purification in chambers do not effectively remove microorganisms from surfaces, especially in difficult to reach areas. Microorganisms settled on surfaces are detached because of air movement in the chamber and can reform bioaerosol. Therefore, for many years, research has been aimed at developing an effective technique for decontaminating air and surfaces in enclosed spaces. In recent years, there has been a development of chemical disinfection techniques based on the generation of fog from biocidal agents. The paper presents selected results of original research, demonstrating that the decontamination of experimental chambers for bioaerosol studies using dry fog generated from hypochlorous acid (HOCl) at a concentration of 300 ppm allows a high degree of microbiological purity before biological experiments and does not pose a threat to human health. Furthermore, the use of HOCl-generated dry fog as a method to decontaminate experimental chambers and laboratory spaces can also reduce the threat posed by biological agents to research personnel and protect against the contamination of the surrounding environment.

Keywords: fog disinfection, bioaerosol, experimental chamber

* **Corresponding author:** E-mail address: (rafal.lewandowski@wat.edu.pl) Rafał B. LEWANDOWSKI

https://dx.doi.org/10.37105/iboa.239
Received 01 November 2024
Available online 23 December 2024
ISSN 2450-1859, eISSN 2450-8721
Published by Centrum Rzeczoznawstwa Budowlanego

1 Introduction

Conducting research using bioaerosols containing live microorganisms requires the use of closed experimental systems equipped with various types of safeguards against the uncontrolled release of biological agents into the surrounding environment.

The purpose of decontaminating the interior of the chamber (air and solid surfaces) carried out before the start of experimental research is to ensure appropriate microbiological cleanliness for biological studies. The effectiveness of the disinfection carried out at this stage has a direct impact on the quality of the results obtained in these studies. On the other hand, decontamination of the chamber's interior, carried out after the experiments are completed using a disinfectant with confirmed biocidal activity, should ensure safety for the laboratory personnel and prevent contamination of the surrounding environment.

For this reason, research has been ongoing for many years to develop an effective technique to decontaminate air and surfaces in enclosed spaces, allowing for high antimicrobial efficacy. At the same time, the biocidal agent used for decontamination must be safe for humans and the surrounding environment. This work presents selected issues related to bioaerosol research in experimental chambers and the possibilities of effective inactivation of microorganisms, as well as selected results of the authors' own research in this area.

2 Bioaerososls

An aerosol in which the dispersing medium is air (or another gas) and the dispersed phase consists of biological agents is referred to as a biological aerosol or bioaerosol. Biological particles represent a small percentage (<0.1%) of all molecules in naturally occurring aerosols. Natural aerosols consist of particles of varying sizes – from sub-micrometer particles to large droplets with diameters exceeding thousands of microns. In the natural environment, airborne microorganisms rarely occur as single cells. They are transported in the air in the form of clusters or aggregates, or as particles settled on inorganic particles (Roszak and Colwell, 1987; Griffin, 2007). An example of aggregates formed by the endospores of Bacillus atrophaeus is presented in Figure 1.

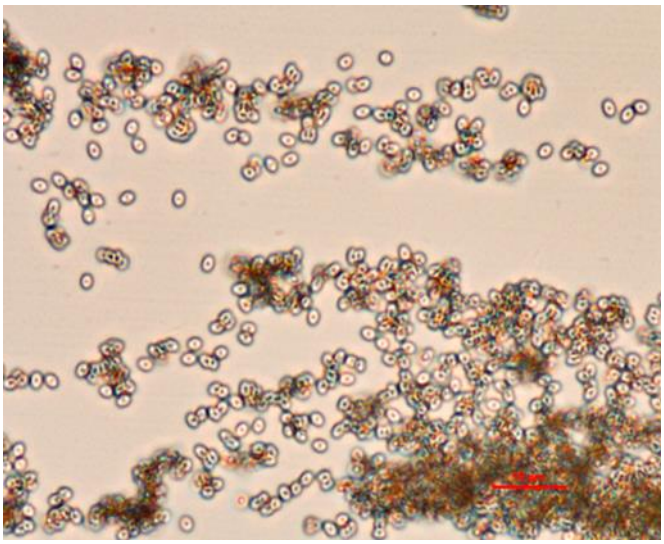

Figure 1. *Aggregates of endospores of Bacillus atrophaeus formed in the experimental chamber (from their own research).*

On dust particles, microorganisms can also form biofilm structures, which are clusters of bacteria surrounded by mucus (Alimova et al., 2007). This ensures a significantly lower susceptibility of microorganisms to harmful environmental factors, such as UV radiation or drying out. In such conditions, microorganisms maintain their virulence for several days, or even several dozens of days, and with even slight air movements occurring in constantly closed rooms, they can repeatedly rise and fall onto surfaces (the so-called phenomenon of reaerosolization), thereby creating a continuous risk of reinfection (Paton et al., 2015). More than 40% of the particles in aerosols, including microbial cells, have a diameter greater than 7 μm (Lighthart, 1997a). Pseudomonas syringae and Pantoea agglomerans live 2.4 to 4.1 times longer in particles with a large aerodynamic diameter (7 μm) than in particles with

a small aerodynamic diameter (1.1 μm) (Lighthart and Shaffer, 1997b). Such large particles (with a diameter of 12 μm) can be filled with over two thousand endospores of the genus Bacillus or vegetative cells of Gram-negative bacteria. They pose a significant threat to the health of the population due to the considerably higher infectious potential of even a small number of such particles, absorbed from the air into the upper respiratory tracts of humans and animals (Thomas et al., 2008).

Furthermore, bioaerosol particles can remain suspended in the air for many hours, and if they are not effectively inactivated in a short period, their concentration can increase over time and pose a greater threat (Marthi et al., 1990; Paton et al. 2015). Among the biologically derived particles, bacterial endospores and fungal spores are particularly adapted for airborne dispersal because these forms of microorganisms are resistant to drying out. Therefore, for many years, research has been aimed at developing a highly efficient, cost-effective, and safe method to rid the indoor air of harmful biological factors.

3 Experimental chambers for bioaerosol research

The controlled and safe generation of aerosols containing biological agents can only be conducted under laboratory conditions within highly sealed experimental setups. The most important element of these systems is usually special chambers, whose design should meet the conditions specified for Class III microbiological safety cabinets (PN-EN 12469:2002). In the global literature, one can find descriptions of many experimental setups used for bioaerosol research, which vary in size, shape, and degree of airtightness of the laboratory in which they are installed (Agranovski et al., 2003; Deloge-Abarkan et al., 2007). The simplest and smallest research setups take the form of chambers, while the most complex ones have dimensions corresponding to the size of a room or even a residential building (Cheng et al., 1999; Buttner et al., 2001; Doussin et al. 2023). In basic research conducted under laboratory conditions, chambers with a capacity ranging from several dozen to several thousand liters are typically used (most often around 500 liters), and made from various materials that have high resistance to physical and chemical factors (Heildelberg et al., 1997; Doussin et al., 2023). The most used materials are transparent plastics, stainless steel, or aluminum (Doussin et al., 2023).

The chambers are equipped with various devices that are used, among other things, to measure the pressure, temperature, and humidity of the indoor air (Agranowski et al., 2003). Small laboratory chambers usually have special gloves that allow the safe performance of various tasks inside the chamber by individuals outside (Macher et al., 2008; Horve et al., 2021). An example of a small experimental chamber for bioaerosol research used in our earlier studies is shown in Figure 2.

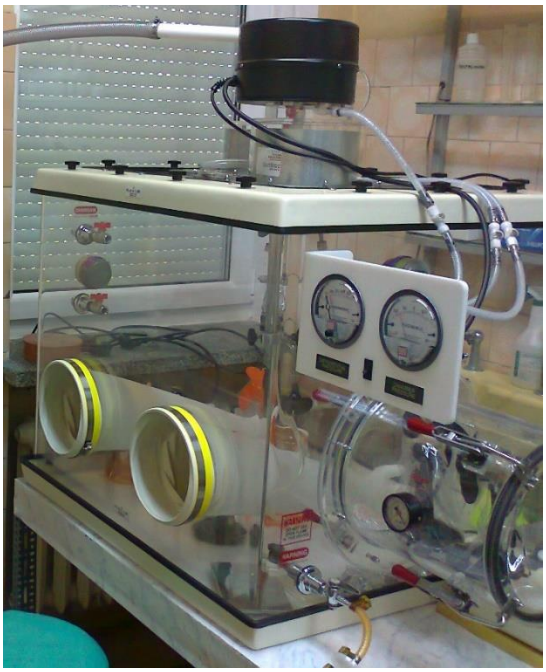

Figure 2. *A small experimental chamber for bioaerosol studies (source: own research (Lewandowski et al., 2010)).*

Furthermore, depending on the category of pathogenic microorganisms intended for use (according to the classification of pathogenic organisms for humans), the entire experimental setup must be in a laboratory room that meets the appropriate level of airtightness (PN-EN 12128:2000).

4 Selected chemical methods for inactivation of bioaerosols in experimental chambers

Physical and chemical methods can be used for the disinfection and sterilization of closed experimental systems used for bioaerosol research (Duchaine, 2016). Most often, various chemical compounds are used, which can be utilized in the form of solutions, as well as in aerosol or gaseous form. The chemical disinfection of surfaces performed using wetting or immersion techniques can be applied only to the movable elements of experimental setups or to permanent horizontal surfaces located at the bottom of chambers. That disinfection technique does not find application for the disinfection of other elements of experimental setups (including hard-to-reach places) and for the inactivation of biological agents suspended in the air inside the chamber.

Therefore, in recent years, there has been a development of chemical disinfection techniques based on fogging or the generation of gases with biocidal properties. Obtaining a stable number of particles with a diameter of <10 μm during the disinfection process corresponds to the fraction known as dry fog. This is important because such a state allows disinfectant particles to remain in the air for a longer period, thereby increasing the effectiveness of inactivating microorganisms in the air and on surfaces, as well as eliminating potential surface dampness. In the fogging process, the most used substances are hydrogen peroxide, hypochlorous acid, peracetic acid, dodecyl dimethylammonium chloride, isopropanol, and ozone. The effectiveness of the antimicrobial action of these substances has been assessed in numerous independent studies. In addition, a series of studies have been conducted to determine the harmful effects on various equipment, devices, and humans associated with the use of these methods (Rutala et al., 2023). Data from these studies have had a significant impact in recent years on users' choice of the appropriate method for air and surface disinfection. The most used methods involve gaseous hydrogen peroxide or a dry fog of hypochlorous acid. However, the safe and effective use of these techniques often requires expensive and specialized equipment, which should only be operated by experienced and trained personnel. Currently, there are many devices based on these techniques available, but each has its specific characteristics, which is why it is necessary to select the appropriate process parameters in advance and to test the device's effectiveness under various conditions.

4.1 Decontamination using hydrogen peroxide gas

Hydrogen peroxide belongs to the group of peroxide agents and has a wide range of applications as an antiseptic, disinfectant and sterilizing agent (Ayub et al., 2024). It is a low-toxic agent that naturally decomposes in the environment into oxygen and water. At room temperature (25ºC) and normal atmospheric pressure (101.35 kPa), it exists in liquid form and has antibacterial properties. For skin disinfection, the use of 3% hydrogen peroxide, commonly known as a hydrogen peroxide solution, is sufficient. On the other hand, for the sterilization of surfaces in enclosed spaces contaminated with microorganisms, it is necessary to use significantly higher concentrations of liquid hydrogen peroxide (10 – 30%), since only then can the highest bactericidal effectiveness be achieved, and spore forms of bacteria be eliminated. However, it is not safe as concentrated liquid hydrogen peroxide is an unstable compound that has explosive and self-igniting properties and causes material corrosion (Mostak, 2008; Wirtanen, and Salo, 2003). The use of the gaseous form of hydrogen peroxide has been shown to be much safer as the same bactericidal effect can be achieved at concentrations significantly lower than those of the liquid form. Therefore, automatic systems that use vaporized hydrogen peroxide (VHP) have been developed for the sterilization of various types of enclosed spaces (McEvoy and Rowan, 2019). The example device VHP 1000 ED - EDS 230 to generate VHP is shown in Figure 3.

Figure 3. *Steris VHP 1000 ED - EDS 230 Vaporised Hydrogen Peroxide Generator.*
(sorce: www.equipnet.com)

The VHP is generated by the vaporization of liquid hydrogen peroxide at a temperature of 120°C, and its concentration is maintained below the condensation point, the value of which depends on the ambient temperature. During the sterilization of enclosed spaces (for example, rooms, experimental chambers), the concentration of VHP is generally maintained well below the saturation level (0.1 – 1.5 mg/l at 25ºC), while in the case of sterilizing medical devices, higher concentrations can be used while maintaining a higher process temperature. In the case of using VHP at a concentration higher than the saturation point for a given temperature, hydrogen peroxide condensation forms on surfaces, which can cause damage to certain materials. To avoid the occurrence of this unfavorable phenomenon, it is necessary to properly develop the process cycle for the given room, considering the temperature and volume of the sterilized space. The sterilization method using VHP is a "dry" process, in which the concentration of the active substance is significantly lower than that in methods based on oxidizing liquids (e.g. peracetic acid).

The VHP method demonstrates high bactericidal, virucidal, fungicidal, and sporicidal efficacy (Dutt et al., 2004, Ayub et al., 2024). The mechanism of action of VHP is primarily based on the generation of highly reactive hydroxyl radicals that attack nucleic acids and proteins, leading to the destruction of various structural elements of microorganisms. In vapor form, hydrogen peroxide is significantly more effective in denaturing proteins compared to its liquid form (Finnegan et al., 2010). Unlike the liquid form of hydrogen peroxide, the gaseous state exhibits very rapid sporicidal activity even at very low concentrations (around 0.1 mg/l) (Rogers et al., 2005).

A very important advantage of the VHP method is the safety of the chemical compound used for the environment (rapid decomposition into oxygen and water vapor) and its low level of harm to humans. However, direct contact with VHP at a biocidal concentration (> 0.1 mg/l) should be avoided. It is widely accepted that the safe concentration of VHP for personnel, allowing prolonged contact with the agent, is 1 ppm for 8 hours, while the level of short-term exposure is 75 ppm for 30 minutes. Gaseous hydrogen peroxide is an odorless and colorless gas; however, concentrations above 1 ppm have a distinctly irritating effect. Simple devices are available to monitor peroxide levels in a given area.

Gas sterilization processes can be divided into vacuum and atmospheric pressure processes. VHP systems operating under atmospheric pressure provide rapid, low-temperature sterilization of contaminated enclosed spaces. Systems of this kind are commonly used to destroy microorganisms suspended in the air and those present on various surfaces. They are used, among other places, in healthcare facilities, diagnostic and research laboratories,

pharmaceutical companies, animal facilities, etc. The typical VHP cycle under atmospheric pressure consists of four phases: dehumidification, saturation, sterilization, and aeration. The course of each phase is automatically controlled and monitored. The automated decontamination cycle allows for real-time recording of cycle parameters and validation. However, vacuum systems with VHP provide greater gas penetration in the case of packaged medical equipment, centrifuges, and freeze dryers. In recent years, this technology has been applied to the low-temperature sterilization of packaged medical products. A typical vacuum sterilization cycle using VHP includes leak testing, saturation, sterilization, and aeration.

4.2 Decontamination using a dry fog of hypochlorous acid

In recent years, many experimental studies have demonstrated the high antimicrobial efficacy of HOCl used for air and surface disinfection by fogging in enclosed spaces (Fukuzaki, 2023). This acid stands out among many biocidal agents due to its high antimicrobial efficacy and broad spectrum of action against various groups of pathogens: bacteria, viruses, and fungi, including spore forms. It has been shown to be 80 times more effective in destroying microorganisms than sodium hypochlorite (da Cruz Nizer et al., 2020). HOCl easily penetrates the bacterial cell wall and, unlike sodium hypochlorite and hydrogen peroxide, is nonirritating and does not cause toxic or allergic reactions at the most effective antimicrobial concentrations. At concentrations close to 200 ppm, hypochlorous acid is considered safe and nontoxic (Gökçe et al., 2023). In studies conducted at the Biomedical Engineering Centre of the Institute of Optoelectroncs at the Military University of Technology (MUT), in an experimental chamber designed for experiments using dry fog of HOCl, it was shown that four exposures of human fibroblasts to aerosolized HOCl at a concentration of 300 ppm for 3 minutes did not cause significant cytotoxicity and genotoxic effects. However, exposure of cells to aerosolized HOCl at a concentration of 500 ppm for 5 minutes caused analogous damage (Lewandowski et al., 2024).

For this reason, considering its high antimicrobial efficacy and safety for humans, hypochlorous acid in the form of a dry fog is increasingly used to decontaminate indoor air in various enclosed spaces (e.g., hospitals, food industries, agriculture, and others). This method can also be used in laboratories engaged in bioaerosol research to decontaminate experimental chambers and laboratory rooms (Norkaew et al., 2024). It is important to carry out effective decontamination of the chamber both before the start of the research and after its completion. In the first case, fogging decontamination helps in the inactivation of accidental microbiological contaminants, the presence of which could lead to the distortion of results in further studies using sterile biological materials (e.g., cell cultures). However, decontamination after the completion of experiments is one of the elements that ensures safety in laboratories conducting research using biological materials in experimental chambers. During these studies, a threat to research staff can arise from both the intentional release of bioaerosols generated for research purposes and the uncontrolled generation of bioaerosols from contaminated surfaces within the chamber or materials used in the studies.

In the studies mentioned above conducted at the Biomedical Engineering Centre of the Institute of Optoelectronics at MUT, a microbiological assessment of the effectiveness of decontamination of the experimental chamber using a dry fog of HOCl was also carried out. (Figure 4).

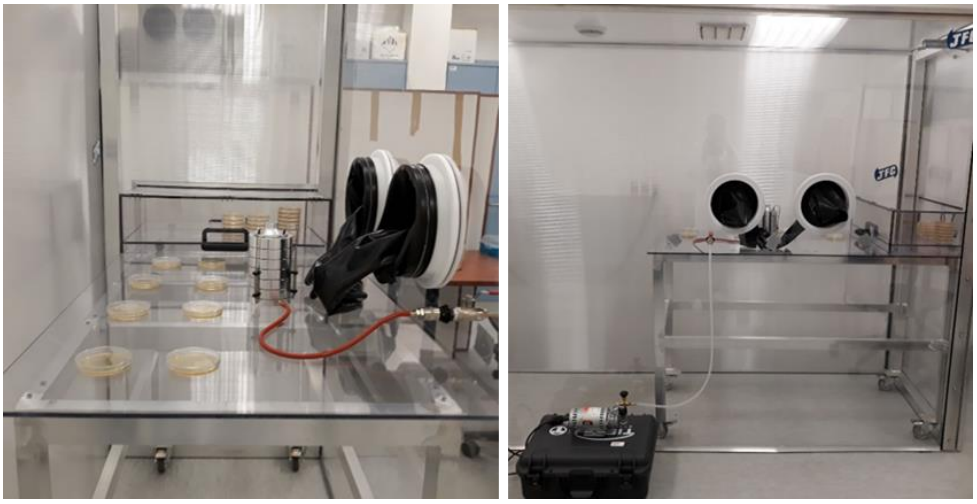

Figure 4. *Microbiological studies evaluating the effectiveness of HOCl decontamination in the experimental chamber.*

The reduction in the number of microorganisms in the air within the chamber was assessed using the impact method with a six-stage Andersen cascade type impactor TE-10-800 (Tisch-Environmental Inc., Ohio, USA) for 30 minutes, at an air flow rate through the sampler of 28.3 l/minute. (Figure 5).

Figure 5. *Six-stage Andersen cascade impactor type TE-10-800 (source: own research).*

In the first stage of the investigation, the total number of bacteria in the air inside the chamber was measured before decontamination (the so-called "ambient air") i.e., the microbiological background of the chamber's interior. In the second stage, the effectiveness of decontamination of the chamber after fogging with HOCl (300 ppm (BMAsept surf&air, BioMedAqua, Dębica, Poland) for 5 minutes) was checked, and then the fogging chamber was left for 60 minutes. After that time, indoor air samples were collected using an Andersen impactor. Inside the sampler, Petri dishes with nutrient agar were placed at various levels. After the sampling was completed, the plates were incubated at a temperature of 35°C for 3 days, and the colonies grown were counted. Due to the characteristic nature of this type of sampler and the overlap of colonies formed from the deposition of individual vegetative bacterial cells in one spot on the surface of solid media at higher microbial densities in bioaerosols, appropriate correction tables were used to determine the most probable number of microorganisms in the air (Andersen, 1958). The results were presented as the number of bacterial colonies that grew at all six levels of the sampler, after accounting for the appropriate correction factor, and were expressed as colony-forming units (CFU).

After decontaminating the chamber with dry fog HOCl, a reduction of 87.63% in the total number of viable culturable bacteria compared to the initial bacterial count in the chamber was observed. Furthermore, it was found that before decontamination, airborne microorganisms in the chamber predominantly had aerodynamic diameters in the ranges of 2.0 - 1.1 and 1.0 - 0.65 µm, with their numbers decreasing by 94.83% and 82.61% after decontamination, respectively (Figure 6).

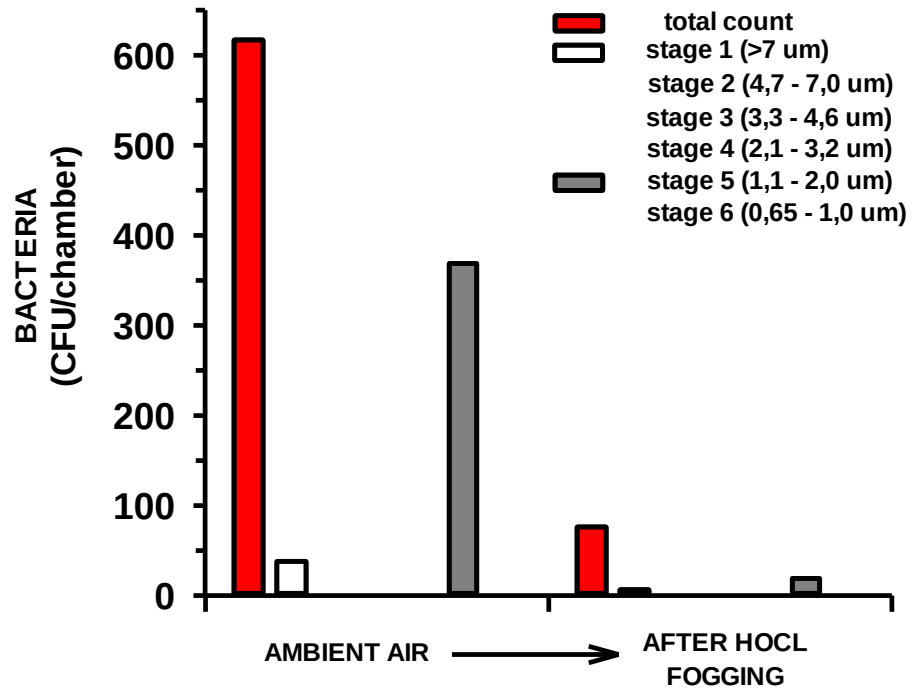

Figure 6. *Effectiveness of indoor air purification in the experimental chamber after application of HOCl dry fog (300 ppm for 60 min.). Assessment of the number of viable cultivable microorganisms in indoor air was carried out using a six-stage Andersen impactor.*

Based on the results of these studies, it was concluded that decontamination of the chamber with a dry fog of HOCL (300 ppm for 5 minutes), followed by exposure to the fog for 60 minutes, allows microbiological cleanliness to be achieved inside the chamber suitable for research involving cell lines.

5 Conclusions

Previously used decontamination methods that use chemicals generated in aerosol form do not always achieve high antimicrobial effectiveness and often have an adverse effect on people and equipment. Technologies for generating disinfectants in the form of dry fog, developed in recent years, ensure effective inactivation of microorganisms in the air and on surfaces, but they are not always cost-effective and require operation by professional personnel. Decontamination within experimental chambers for bioaerosol research using dry fog generated from HOCl at a concentration of 300 ppm allows for appropriate microbiological cleanliness before biological experiments and does not pose a threat to human health. The use of HOCl-generated dry fog as a method for decontaminating experimental chambers and laboratory spaces can enhance the quality of biological research conducted and reduce the threat posed by biological agents to research personnel.

Acknowledgments

This research was partially funded by the Polish National Centre for Research and Development, contract no. DOB-SZAFIR/02/A/002/02/2021; the project entitled “Mobile disinfection system for medical support of the Polish Armed Forces in counteracting SARS-CoV-2”.

Bibliography

1. Agranovski, V., Ristovski, Z., Hargreaves, M., Blackall, P. J., and Morawska, L. (2003). Performance evaluation of the UVAPS: influence of physiological age of airborne bacteria and bacterial

stress. *Journal of Aerosol Science*, *34*(12), 1711-1727. https://doi.org/10.1016/S0021-8502(03)00191-5

2. Alimova, A., Roberts, M., Katz, A., Rudolph, E., Steiner, J. C., Alfano, R. R., and Gottlieb, P. (2006). Effects of smectite clay on biofilm formation by microorganisms. *Biofilms, 3(1)*, 47-54. https://doi.org/10.1017/S1479050507002128
3. Andersen, A. A. (1958). New sampler for the collection, sizing, and enumeration of viable airborne particles. *Journal of bacteriology*, *76*(5), 471-484. https://doi.org/10.1128%2Fjb.76.5.471-484.1958
4. Ayub, A., Cheong, Y. K., Castro, J. C., Cumberlege, O., and Chrysanthou, A. (2024). Use of Hydrogen Peroxide Vapour for Microbiological Disinfection in Hospital Environments: A Review. *Bioengineering*, *11*(3), 205. https://doi.org/10.3390/bioengineering11030205
5. Buttner, M. P., Cruz-Perez, P., and Stetzenbach, L. D. (2001). Enhanced detection of surface-associated bacteria in indoor environments by quantitative PCR. *Applied and Environmental Microbiology*, *67*(6), 2564-2570. https://doi.org/10.1128/aem.67.6.2564-2570.2001
6. Cheng, Y. S. (1999). Detection of bioaerosols using multiwavelength UV fluorescence spectroscopy. *Aerosol Science and Technology*, *30*(2), 186-201. https://doi.org/10.1080/027868299304778
7. da Cruz Nizer, W. S., Inkovskiy, V., and Overhage, J. (2020). Surviving reactive chlorine stress: responses of gram-negative bacteria to hypochlorous acid. *Microorganisms*, *8*(8), 1220. https://doi.org/10.3390/microorganisms8081220
8. Deloge-Abarkan, M., Ha, T. L., Robine, E., Zmirou-Navier, D., and Mathieu, L. (2007). Detection of airborne Legionella while showering using liquid impingement and fluorescent in situ hybridization (FISH). *Journal of Environmental Monitoring*, *9*(1), 91-97. https://doi.org/10.1039/B610737K
9. Doussin, J. F., Fuchs, H., Kiendler-Scharr, A., Seakins, P., and Wenger, J. (2023). *A Practical Guide to Atmospheric Simulation Chambers* (p. 339). Springer Nature. https://library.oapen.org/handle/20.500.12657/62970
10. Duchaine, C. (2016). Assessing microbial decontamination of indoor air with particular focus on human pathogenic viruses. *American Journal of Infection Control*, *44*(9), S121-S126. https://doi.org/10.1016/j.ajic.2016.06.009
11. Dutt, D. L., Turetsky, A. L., Brickhouse, M., Pfarr, J. W., McVey, I. F., Meilander, S. L., and Dallmier, A. W. (2004). Effectiveness of vaporous hydrogen peroxide for the decontamination of representative military materials. In *Poster FP10, 24th Army Science Conference Proceedings*. https://apps.dtic.mil/sti/citations/ADA449628
12. Finnegan, M., Linley, E., Denyer, S. P., McDonnell, G., Simons, C., and Maillard, J. Y. (2010). Mode of action of hydrogen peroxide and other oxidizing agents: differences between liquid and gas forms. Journal of Antimicrobial Chemotherapy, 65(10), 2108-2115. https://doi.org/10.1093/jac/dkq308
13. Fukuzaki, S. (2023). Uses of gaseous hypochlorous acid for controlling microorganisms in indoor spaces. *Journal of Microorganism Control*, *28*(4), 165-175. https://doi.org/10.4265/jmc.28.4_165
14. Gökçe, O. N., Çetin, K., Taş, Ş., Alkan, S., and Dinç, U. (2023). Hypochlorous Acid: A Novel Agent Against Echinococcus granulosus Protoscoleces. *Infectious Diseases and Clinical Microbiology*, *5*(2), 158. https://doi.org/10.36519%2Fidcm.2023.238
15. Griffin, D. W. (2007). Atmospheric movement of microorganisms in clouds of desert dust and implications for human health. *Clinical microbiology reviews*, *20*(3), 459-477. https://doi.org/10.1128%2FCMR.00039-06
16. Heidelberg, J. F., Shahamat, M., Levin, M., Rahman, I., Stelma, G., Grim, C., and Colwell, R. R. (1997). Effect of aerosolization on culturability and viability of gram-negative bacteria. *Applied and Environmental Microbiology*, *63*(9), 3585-3588. https://doi.org/10.1128%2Faem.63.9.3585-3588.1997
17. Horve, P. F., Dietz, L., Northcutt, D., Stenson, J., and Van Den Wymelenberg, K. (2021). Evaluation of a bioaerosol sampler for indoor environmental surveillance of Severe Acute Respiratory Syndrome Coronavirus 2. *PLoS One*, *16*(11), e0257689. https://doi.org/10.1371/journal.pone.0257689
18. Lewandowski, R., Kozłowska, K., Szpakowska, M., Stępińska, M., and Trafny, E. A. (2010). Use of a foam spatula for sampling surfaces after bioaerosol deposition. *Applied and Environmental Microbiology*, *76*(3), 688-694. https://doi.org/10.1128%2FAEM.01849-09

19. Lewandowski, R. B., Stępińska, M., Osuchowski, Ł., Kasprzycka, W., Dobrzyńska, M., Mierczyk, Z., and Trafny, E. A. (2024). The HOCl dry fog–is it safe for human cells?. *Plos one*, *19*(5), e0304602. https://doi.org/10.1371/journal.pone.0304602
20. Lighthart, B. (1997a). The ecology of bacteria in the alfresco atmosphere. *FEMS microbiology ecology*, *23*(4), 263-274. https://doi.org/10.1111/j.1574-6941.1997.tb00408.x
21. Lighthart, B., and Shaffer, B. T. (1997b). Increased airborne bacterial survival as a function of particle content and size. *Aerosol Science and Technology*, *27*(3), 439-446. https://doi.org/10.1080/02786829708965483
22. Macher, J., Chen, B., and Rao, C. (2008). Chamber evaluation of a personal, bioaerosol cyclone sampler. Journal of Occupational and Environmental Hygiene, 5(11), 702-712. https://doi.org/10.1080/15459620802380351
23. Marthi, B., Fieland, V. P., Walter, M., and Seidler, R. J. (1990). Survival of bacteria during aerosolization. *Applied and environmental microbiology*, *56*(11), 3463-3467. https://doi.org/10.1128%2Faem.56.11.3463-3467.1990
24. McEvoy, B., and Rowan, N. J. (2019). Terminal sterilization of medical devices using vaporized hydrogen peroxide: a review of current methods and emerging opportunities. *Journal of applied microbiology*, *127*(5), 1403-1420. https://doi.org/10.1111/jam.14412
25. Mostak, P. (2008). Chemistry and properties of liquid explosives. In: *Detection of Liquid Explosives and Flammable Agents in Connection with Terrorism* (pp. 15-25). Springer Netherlands. http://dx.doi.org/10.1007/978-1-4020-8466-9
26. Norkaew, S., Narikawa, S., Nagashima, U., Uemura, R., and Noda, J. (2024). Efficacy of treating bacterial bioaerosols with weakly acidic hypochlorous water: A simulation chamber study. *Heliyon*, *10*(5). https://doi.org/10.1016/j.heliyon.2024.e26574
27. Paton, S., Thompson, K. A., Parks, S. R., and Bennett, A. M. (2015). Reaerosolization of spores from flooring surfaces to assess the risk of dissemination and transmission of infections. *Applied and Environmental Microbiology*, *81*(15), 4914-4919. https://doi.org/10.1128/aem.00412-15
28. Polska Norma PN-EN 12128:2000, Laboratoria badawcze, rozwoju i analizy. Stopnie hermetyczności laboratoriów mikrobiologicznych, strefy ryzyka i wymagania względem lokalizacji i bezpieczeństwa fizycznego.
29. Polska Norma PN-EN 12469:2002, Biotechnologia. Kryteria działania komór bezpiecznej pracy mikrobiologicznej.
30. Rogers, J. V., Sabourin, C. L. K., Choi, Y. W., Richter, W. R., Rudnicki, D. C., Riggs, K. B., and Chang, J. (2005). Decontamination assessment of Bacillus anthracis, Bacillus subtilis, and Geobacillus stearothermophilus spores on indoor surfaces using a hydrogen peroxide gas generator. *Journal of applied microbiology*, *99*(4), 739-748. https://doi.org/10.1111/j.1365-2672.2005.02686.x
31. Roszak, D. B., and Colwell, R. (1987). Survival strategies of bacteria in the natural environment. *Microbiological reviews*, *51*(3), 365-379. https://doi.org/10.1128%2Fmr.51.3.365-379.1987
32. Rutala, W. A., Boyce J. M., and Weber, D. J. (2023). Disinfection, sterilization, and antisepsis: an overview. *American journal of infection control*, *51(11S)*:A3-A12. https://doi.org/10.1016/j.ajic.2023.01.001
33. Thomas, R. J., Webber, D., Sellors, W., Collinge, A., Frost, A., Stagg, A. J., and Titball, R. W. (2008). Characterization and deposition of respirable large-and small-particle bioaerosols. *Applied and environmental microbiology*, *74*(20), 6437-6443. https://doi.org/10.1128%2FAEM.01194-08
1. Wirtanen, G., and Salo, S. (2003). Disinfection in food processing–efficacy testing of disinfectants. *Reviews in Environmental Science and Biotechnology*, *2*, 293-306. http://dx.doi.org/10.1023/B:RESB.0000040471.15700.03