Changes in the composition of the indoor bioaerosol as a result of the ozonation process carried out under different ventilation air flow rate

Zmiany składu bioaerozolu w pomieszczeniu w wyniku procesu ozonowania prowadzonego przy różnych wartościach strumienia wentylacji

WALTER MUCHA, EWA ZABŁOCKA-GODLEWSKA, ALEKSANDRA MIERZEJOWSKA, ALEX BORKOWSKI, ALEKSANDRA BIENIEK, KINGA BISKUP, KAMIL FOIT, KAROLINA HULBOJ, KAMIL KRAWCZYK

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The microbial composition of indoor bioaerosol is very important for the health and well-being of occupants of these spaces. The efficiency of the ventilation system is crucial in providing proper quality of that. The study aimed to assess the impact of the ozonation carried out under a changeable ventilation air flow rate on airborne microflora. Studies were conducted in a chemical laboratory with four-level force mechanical ventilation. An ozone generator with an efficiency of 1000 g O_3/min was used. The impact method was used for the sampling of airborne bacteria and fungi. The number of psychrophilic, mesophilic bacteria and fungi was estimated as CFU/m^3. For the growth of bacteria and fungi TSA and Sabouraud medium was used respectively. Temperature, relative humidity (RH), volumetric air flow rate (VFR), and ozone concentration were measured. The average minimum ozone concentration reached after 20 minutes of work of one ozone generator was 0.23 ppm (VFR 0.036 m^3/s) and the average maximum concentration was 0.585 ppm (VFR 0.004 m3/s) which resulted in the reduction of number of mesophiles 31.7%, 70.2% and psychrophiles 32.7%, 75.3% respectively. After simultaneous work of two ozone generators the average ozone concentration was 0.96 ppm (VFR 0.122 m^3/s) resulted in disinfection efficiency 43.5% and 9.7% respectively. It was difficult to find any tendency of elimination of fungi. The increase of RH of air cause increase the ozonation efficiency towards the airborne fungi, which was not observed in the case of bacteria. The results of disinfection efficiency carried out in the real conditions showed that the number of the airborne bacteria detected after ozonation depended on the biocidal effect of ozone concentration as well as the value of the VFR and time of exposition.
Keywords: bioaerosol, ozonation, disinfection, air quality, indoor air, mechanical ventilation, airborne bacteria, airborne fungi

Mikrobiologiczny skład bioaerozolu pomieszczeń istotnie wpływa na zdrowie i dobre samopoczucie ich użytkowników. Kluczowe znaczenie w utrzymaniu wysokiej jakości powietrza odgrywa sprawność wentylacji. Celem pracy była ocena wpływu ozonowania prowadzonego przy zmiennym natężeniu strumienia wentylacji na skład mikroflory powietrza. Badania przeprowadzono w laboratorium chemicznym z czterostopniową wymuszoną wentylacją mechaniczną. Zastosowano generator ozonu o wydajności 1000 g O_3/min. Próby bioaerozolu bakteryjnego i grzybowego pobierano metodą zderzeniową. Do hodowli bakterii mezofilnych i psychrofilnych wykorzystano podłoże TSA, grzybów agar Sabouraud. Stężenie mikroorganizmów w powietrzu podano jako CFU/m^3 powietrza. W trakcie badań mierzono temperaturę, wilgotność względną (RH), objętościowe natężenie przepływu powietrza (VFR) i stężenie ozonu. Średnie minimalne stężenie ozonu osiągnięte po 20 minutach pracy jednego generatora ozonu wyniosło 0,23 ppm (VFR 0,036 m^3/s), a średnie maksymalne 0,585 ppm (VFR 0,004 m^3/s), co spowodowało spadek liczebności odpowiednio mezofili o 31,7%, 70,2% i psychrofili o 32,7%, 75,3%. Jednoczesne użycie dwóch generatorów ozonu pozwoliło na uzyskanie średniego stężenie ozonu na poziomie 0,96 ppm (VFR 0,122 m^3/s), co przełożyło się na skuteczność dezynfekcji odpowiednio 43,5% i 9,7%. Trudno było stwierdzić jakiekolwiek tendencje w eliminacji grzybów. Stwierdzono jednak, że wraz ze wzrostem wilgotności względnej powietrza zwiększała się skuteczność ozonowania względem grzybów, czego nie stwierdzono jednoznacznie w przypadku bakterii. Wyniki skuteczności dezynfekcji przeprowadzone w warunkach rzeczywistych wykazały, że stężenie bakterii w powietrzu po ozonowaniu zależało od biobójczego działania stężenia ozonu, wartości VFR i czasu ekspozycji.
Słowa kluczowe: bioaerozol, ozonowanie, dezynfekcja, jakość powietrza, powietrze wewnętrzne, wentylacja mechaniczna, mikroorganizmy przenoszone drogą powietrzną, bakterie, grzyby.

Walter Mucha https://orcid.org/0000-0002-7476-0614 – DSc; The Silesian University of Technology, Faculty of Energy and Environmental Engineering, Department of Air Protection, Gliwice, Poland
Ewa Zabłocka-Godlewska https://orcid.org/0000-0002-4212-8913 – Associate Prof.; The Silesian University of Technology, Faculty of Energy and Environmental Engineering, Department of Air Protection, Gliwice, Poland
Aleksandra Mierzejowska https://orcid.org/0000-0003-0191-5782 – DSc; The Silesian University of Technology, Faculty of Faculty of Mining, Safety Engineering and Industrial Automation, Department of Geoengineering and Raw Materials Extraction, Gliwice, Poland,
Alex Borkowski, Aleksandra Bieniek, Kinga Biskup, Kamil Foit, Karolina Hulboj, Kamil Krawczyk – Student of The Silesian University of Technology, Gliwice, Poland
Adres do korespondencji/Corresponding author: E-mail address: walter.mucha@polsl.pl

Introduction

Ozone (O_3), a three-atom allotrope of oxygen, has low molecular stability and is one of the strongest oxidizing agents. It occurs in nature, and its concentration in the atmosphere is about 0.04 ppm [1]. Its rate of decomposition increases with increasing temperature. At high concentrations, it decomposes explosively [2]. In the gas phase, ozone has a half-life of about 20 minutes [3]. It is a powerful oxidizing agent that causes changes in the molecular structures of complex cell membrane compounds, which are responsible for the integrity of bacteria, protists, fungal cells and viral particles [4]. Oxidation of amino acids, phospholipids, glycolipids, glycoproteins and polyunsaturated fatty acids leads to disruption of cell membrane function and stability, leakage of cell components, and ultimately cell death. Ozone radicals, generated during ozone breakdown, also induce cell lysis by penetrating the cell membrane and altering cellular metabolism. Intracellular damage processes also occur through protein oxidation, DNA damage and disruption of enzymatic activity [5], [6], [7]. All this leads to the destruction of microorganisms exposed to ozone. This gas is evenly distributed throughout the room and can effectively penetrate hard-to-reach areas, including small crevices. Ozone is considered an inexpensive and somewhat environmentally friendly disinfectant, as its use reduces the use of other potentially harmful chemical disinfectants. As a highly unstable substance that readily converts to molecular oxygen, it leaves few harmful residues [8]. In addition, unlike antibiotics, ozone's biocidal mechanism prevents the development of microbial resistance [2]. Ozone is widely used in air and surface disinfection, e.g. in surgical wards, in the food and pharmaceutical industry, and in water treatment [9], [10]. It is used on a massive scale in the disinfection of heating, ventilation and air conditioning systems [11]. The most problematic factor in ozone disinfection in such installations is to control its concentration and keep it within the desired range (0.055 ppm) [8], [12]. It has been proven that treating fruits, vegetables and other foods with ozone extends their shelf life [13]. The ozone concentration for effective biocidal action is 13 mg/m^3 of air [5]. Antimicrobial resistance is on the rise, making common nosocomial infections (e.g. pneumonia, tuberculosis, methicillin-resistant *Staphylococcus aureus* (MRSA)) difficult to treat with traditional methods due to multidrug resistance (MDR) and antimicrobial resistance. Ozone has been proposed as an effective disinfection tool to control drug-resistant pathogens and reduce the use and consumption of antibiotics [14]. Piletić et al. (2022) [15] presented the results of ozonation of a recovery room in a hospital. Air samples taken after one hour of exposure at an ozone concentration of 15.71 mg/m^3 showed that the most dominant Gram-positive bacteria of the genera *Micrococcus*, *Staphylococcus* and *Bacillus* were reduced by 33%, 58% and 61%, respectively. The genus *Micrococcus* turned out to be the most resistant. Petry et al [16] showed that 68-90% reduction in viral activity was possible after 1-3 hours of exposure to ozone at concentrations of 0.02 to 0.05 ppm. Fontes et al. [17] studied the effects of different doses of ozone on the growth of the bacteria strain *Escherichia coli* – ATCC:35218, and the oxacillin-sensitive pathogen *Staphylococcus aureus* – ATCC:25923. They showed that doses greater than or equal to 20 µg O_3/ml for 5 min exposition prevented the growth of these bacterial strains. At 15 µg O_3/ml for 5 minutes, bacteria were detected, but their concentrations [CFU/m^3] were low. Tu et al (2020) [18] reported that complete removal of bacteria and fungi in a hospital operating room was achieved using ozone at 5 ppm and an exposure time of 40-60 minutes [3]. The biocidal effect on microorganisms varies depending on their individual characteristics. Viruses have been documented to have similar levels of resistance to ozone as bacteria, and ozone has also been shown to be less effective against fungal spores and bacterial endospores (e.g., *Bacillus* and *Clostridium*) than against vegetative cells [9]. Gram-positive bacteria are less resistant to ozone than Gram-negative bacteria [4]. Some factors affect the effectiveness of ozone as an air disinfectant. These include relative humidity (RH) of the air, temperature (T) and air pollution [7], [19]. Hudson [20] obtained maximum antiviral efficacy at a concentration of 25 ppm ozone for 15 minutes, and humidity followed by a short period of >90% RH. Bioaerosols in dwellings and public buildings such as schools, offices, hospitals, etc. are generated mainly by humans. The microbiological quality of indoor air is very important for the well-being and health of these space users. For a particular type of space, such as laboratories, it can also affect the results of ongoing research. Microbiological air contaminants can contaminate the reagents, biocultures, etc. Proper air quality reduces the risk of spreading airborne infectious diseases, allergies (allergens produced by mold, bacteria) and poisoning (bacterial endotoxins, mycotoxins) [21], [22], [23], [24], [25], [26].

High concentrations of ozone in the air have harmful effects on humans. It should be taken into account that inhaling high concentrations of ozone is dangerous to the respiratory tract and causes damage, shortness of breath, sore throat, eye pain, chest pain and irritation. Therefore, it is important to control the concentration of ozone during the ozonation process [25]. According to the Regulation of the European Parliament and of the Council (EC) No. 1272/2008 [27], the maximum permissible concentration of ozone in the air is 0.15 mg/m^3 (0.076 ppm). For occupational risks, this concentration must not be exceeded for 8 hours a day and 40 hours a week. The lethal concentration for humans is 100 mg O_3/m^3 of air [5].

The aim of the study was to determine the effectiveness of ozone air disinfection under real conditions. Study was conducted in a chemical laboratory, a specific type of indoor space that is rarely studied. Most of the studies are typical case studies. Our research was conducted under real conditions determined by the variable efficiency of the ventilation system, and various temperature and RH values. This approach to the problem allows to monitor the impact of changeable temperature [°C], RH [%], controlled air flow rate [m^3/s] and ozone concentration on the efficiency of ozone treatment and to determine the optimal values of these factors in real conditions. This would make it possible to predict the efficiency of the process and determine the required concentration or time of ozone production under given conditions.

Material and methods

Sampling and measurement series location

The study was carried out in a chemical laboratory. Samples were collected for 6 weeks in the autumn-winter season (November and December). The research focused on the assessment of the effect of the ozonation process carried out at different values of VFR on the composition of the bioaerosol. The laboratory room was 6 m long x 4 m wide x 3 m high (volume 72 m^3). In the laboratory, instead of opening windows, there were ceiling skylights. The laboratory is equipped with a 4-stage mechanical exhaust and supply ventilation system. The air exchange and flow rate can be adjusted. Laboratory equipment is

typical for a basic chemical analysis laboratory (laboratory tables, cabinets, sink, basic apparatus, laboratory glassware, reagents, etc.). The laboratory was used in the normal way and in accordance with its intended purpose, except for the moment when disinfection and measurements were carried out (no one was in the room during this time). The sources of bioaerosol in the laboratory were the users of the laboratory, the air introduced through the ventilation system supply and the probable emission from the sink.

The study was carried out under different ozone concentration conditions, determined by variable VFR values. During the research, 6 measurement series were performed. Details of the series are given in Table 1. The dimensions and ventilation system of the measuring room as well as the arrangement of the equipment used are shown in Fig. 1.

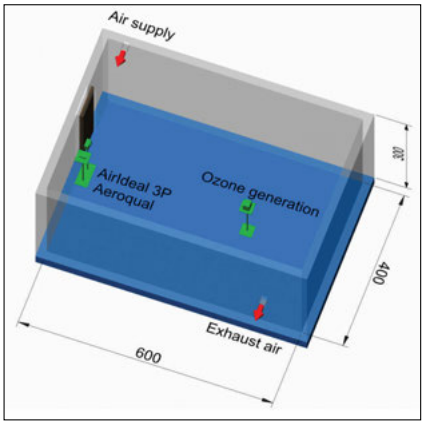

Figure 1.
Dimensions and ventilation system of the measuring room and the arrangement of the equipment used
Rysunek. 1. Wymiary i system wentylacyjny pomieszczenia pomiarowego oraz rozmieszczenie wykorzystanej aparatury

Ozone generation and measurement

A Lehmann Alloy 60,000 mg/h ozone generator was used. In series 1-4 and 6, the amount of ozone produced was 1000 g/min. The exception was series 5, during which two ozone generators operated simultaneously (2000 g/min) to increase the concentration of ozone in the air. The ozone generator was located in the center of a room marked by diagonal lines, at a height of 110 cm above the floor. During the 5th series, both generators used were at the same point, close to each other. The ozone concentration was measured using an Aeroqual Series 500 ozone meter equipped with an interchangeable GSS and GSE sensor (additionally equipped with a Tand RH (HH TRH) sensor). The sensors differ in measurement accuracy: GSS type – range was 0-0.5, minimum detection limit of 0.001 ppm, factory calibration accuracy < ± 0.008 ppm 0-0.1 ppm < ± 10% 0.1-0.5 ppm; , type GSE – range 0-10 ppm, minimum limit of detection 0.01 ppm, factory calibration accuracy < ± 0.01 +7.5%. Taking into account the set value of the ventilation flux and the predicted ozone concentration (based on previous tests – results not presented), an appropriate measuring head (sensor type) was selected for ozone measurement. The ozone meter was 110 cm above the floor, against the wall at a distance of 3 meters from the ozone generator. Measurements were carried out continuously from the time point before ozonation to 30 minutes after the end of emission.

Microbiological analysis

Microbiological analyses of the bioaerosol included estimation of changes in concentrations of mesophilic and psychrophilic bacteria and fungi. Various microbial growth media were used for this purpose. Trypticasein Soy LAB-AGAR (TSA, Biomaxima) was used for bacterial growth and Sabouraud Dextrose LAB-AGAR (SB, Biomaxima) was used for fungi. Samples were collected at three time points, in three replications: before ozonation, immediately after 20 minutes of ozonation, and 30 minutes after the end of emission. The measurements were carried out with the impact method using the AirIdeal 3P Biomerieux sampler (the accuracy of the measuring instrument is 100 ± 6.5 liters per minute). The air sampler was placed 110 cm above the floor (using a tripod) against a wall at a distance of 3 meters from the ozone generator near the location of the ozone sampler. In each series, 100 dm^3 of air was taken per plate.

After sampling, the plates were incubated:

- psychrophilic bacteria at 21 °C for 48-72 h,
- mesophilic bacteria at 36.5°C for 24-48 h,
- fungi at 26°C for 72-96 h.

Colonies were counted using a bacterial colony counter.

The concentration of microorganisms [CFU/m^3] was calculated according to formula 1:

$$A = MPN \times 1000/V \qquad (1)$$

Where:

A – concentration of airborne microorganisms per cubic meter of air [CFU/m^3]
MPN – value calculated on the basis of CFU counting, using FELLER's law (statistical correction),
V – volume of collected air (100 dm^3)

Other measurements

Relative humidity (RH) and air T were measured continuously during the ozonation process and 30 minutes after ozonation. The measurements were performed by using an Aeroqual Series 500 device (equipped with a T I RH (HH TRH) sensor, which was 110 cm above the floor against the wall at a distance of 3 meters from the ozone generator. The average values of these parameters were calculated.

Air flow was measured with an HCA-1 hot wire anemometer in the range of 0.1-2 m/s ; 0.1-20 m/s < ± 5%.The measurement was carried out on the supply grille, the velocity of air outflow was averaged from four measurement points. The volumetric air flow rate (VFR) is calculated using formula 2:

$$\dot{V} = Av \qquad (2)$$

Where:

$\dot{V}$ – volumetric air flow rate [m^3/s]
A – ventilation hole area [m^2]
v – air flow velocity [m/s]

Statistics

Uncertainty in the measurement of volumetric air flow rate.

$$\Delta\dot{V} = \sqrt{\left(\frac{\partial\dot{V}}{\partial A}\cdot\Delta A\right)^2 + \left(\frac{\partial\dot{V}}{\partial v}\cdot\Delta v\right)^2}$$

$$\frac{\partial\dot{V}}{\partial A} = \frac{d}{dA}\cdot Av = v$$

$$\frac{\partial\dot{V}}{\partial v} = \frac{d}{dv}\cdot Av = A$$

Δ – measurement error [m^3/s] $\dot{V}$
$\dot{V}$ – volumetric air flow [m^3/s]
v – air flow velocity [m/s]
A – gap area [m^2]
Δv – anemometer error 0.1 [m/s]
ΔA – area measurement error 0.01 [m^2]

$$\Delta\dot{V} = \sqrt{(v\cdot\Delta A)^2 + (A\cdot\Delta v)^2}$$

Ozone concentration.

The data are presented as mean standard deviation ± (SD) for a 20-minute ozone exposure period.

Results and discussion

Studies of the effectiveness of ozone disinfection were carried out in a chemical

laboratory room that was used in a typical way. During the ozonation and sampling process, there were no people there. It was investigated how variable values of ozone concentration, VFR, temperature and RH affect the effectiveness of disinfection. During the 20-minute ozonation process, the mean minimal ozone concentration achieved during the tests was 0.23 (±0.028) ppm in the 6th series, and the average maximal ozone concentration was 0.96 (±0.041) ppm in the 5th series (Table 1). The effect of ozone on living organisms is proportional to its concentration and duration of exposure, which means that even at low ozone concentrations, microorganisms can be effectively eliminated by increasing the ozone exposition time, which occurs in hospitals [28].

Ozone concentration, volumetric air flow rate and exposure time

In series 1-4 and 6, the ozone generator operated at the same capacity (1000 g O_3/min) for 20 minutes. The exception was series 5, which used two ozone generators, each with a capacity of 1000 g O_3/min. Depending on the VFR setting, the average ozone concentrations over 20 min of ozone treatment varied in a given series. As the VFR of the air increased, the ozone concentration decreased markedly despite the constant rate of ozone production. This, in turn, led to a decrease in the effectiveness of disinfection (Table 1; Figs. 2-7). The detected values ranged from 0.23 O_3 ppm, when the VFR value was 0.136 m^3/s to 0.572 O_3 ppm, when the VFR was 0.004 m^3/s. The highest ozone concentration was found in series 5 (0.96 ppm), although the VFR value was 0.122 m^3/s, which was the result of the operation of two ozone generators (Table 1, Figs. 2-7). Despite the constant time of ozone generation (20 minutes) its concentration, as well as the duration of exposure of microorganisms, differed in a given series. In series 1 and 2 with the lowest VFR value (0.004 m^3/s), the maximal ozone concentration were reached (0.572 and 0.585 ppm, respectively), and were maintained for another 5 minutes after the ozonation process was completed, and then decreased drastically (Fig. 2-3). Thus, the duration of exposure to high concentrations of ozone was the longest in these series (Table 1, Figs. 2-3). The resulting decrease in the number of mesophils and psychrophiles was the greatest in these series and 30 minutes after the end of ozonation it was 71.0%, 28.0% and 53.0%, 50.0% respectively (immediately after ozonation it was 69.8%, 10.0%, and 70.2%, 75.3%, respectively) (Table 1, Figs. 2-3). The decrease in disinfection efficacy observed in the 2nd series 30 minutes after the end of ozonation in relation to the background (mesophiles from 70.2 to 53.0% and psychrophiles from 75.3 to 50.0%) could have been caused by the supply of bacteria through the ventilation air stream or from the sink. Piletić et al. (2022) [15]proved that a sink can be a source of bioaerosol. Misawa et al. (2023) [28] proved the effect of the duration of ozonation on the efficiency of bacterial elimination. The study was conducted in a hermetically sealed chamber with a gaseous ozone generator, a gaseous ozone monitor and a thermohygrometric probe. Two strains of *M. avium* and *M. intracellulare* were exposed to 1 ppm ozone concentrations for 60, 120 and 180 minutes, resulting in reductions: 66.0%, 97.0%, 98.2% for *M. avium*, and 85.8%, 96.3%, and 99.3% for *M. intracellulare*, respectively. In our real conditions studies in series 1 and 2, the ozone concentration was about twice lower, the exposure time was 3 to 9 times lower than in Misawa's (2023) [28] studies (Table 1, Figs. 2-3). Despite of these we obtained results comparable to or slightly lower than the Misawa (2023) [28] results achieved after 60 minutes of exposure for an ozone concentration of 1 ppm. It should be noted, however, that we determined changes in the total number of bacteria (including mesophiles and psychrophiles) and fungi, not individual species. Ozone concentrations (0.96 ppm) were similar to those used by Misawa (2023) [28] in series 5 (Table 1, Fig. 6), but the elimination of bacteria after 20 minutes of ozonation was much lower (psychrophiles 9.7% and mesophiles 43.5%, while 30 minutes after ozone formation obtained values were 41.0% and 43.0%, respectively). Despite the almost doubling of the average ozone concentration in series 5 compared to series 1 and 2 (0.96 ppm, 0.572 ppm and 0.585 ppm, respectively), the reduction in the number of both bacterial groups was smaller, which is likely due to the significantly higher VFR of the air in series 5 (0.122 m^3/s) and the resulting inflow of airborne microorganisms with fresh outside air. Piletić et al. (2022) [15]investigated changes in the total number of bacteria in hospital air after the ozonation process (ozone concentration of 15.71 mg/m^3 for 1 hour). The tests were carried out in the recovery room (cubic capacity of 32.4 m3), where all ventilation openings were sealed before ozonation and the central ventilation system was turned off. With a smaller room volume, higher ozone and time of exposure, and lack of ventilation, the bacterial removal results were similar to those obtained by us in the 1st and 2nd series with the lowest VFR value (0.004 m^3/s). After the ozonation process, the disinfection efficiency achieved by Piletić (2022) [15] was 33% for the point next to the sink, 58% on the desk and 61% on the windowsill what pointed out the sink as a potential source of bioaerosol. Epelle et al. (2022) [29] investigated the effectiveness of ozonation in a closed disinfection chamber using bacteria strains *E. coli* and *S. aureus* and fungi *C. albicans* and *A. fumigatus*. Microorganisms were exposed to ozone at a concentration of 2 ppm for 4, 8, 12 and 16 minutes. The reductions of concentration of *E. coli* were 87%, 94%, 97%, 93%, *S. aureus* 99.5%, 99.7%, 99.5%, 99.9%, *C. albicans* 28%, 22%, 22%, 53%, and *A. fumigatus* 10%, 2%, 7%, 35%, respectively. Increasing ozone concentrations generally resulted in better microbial reduction. An ozone concentration of 10 ppm provided a better reduction of [29] *E. coli* (100% after 16 minutes) compared to values obtained at a concentration of 2 ppm (93% after 16 minutes). The same trend was observed in yeast, where an ozone concentration of 10 ppm for 16 minutes resulted in a 90% reduction in *C. albicans* number, while at 2 ppm only 53%. Complete removal of both groups of microorganisms was achieved when the ozone concentration was 20 ppm and the exposure time was 4 minutes. The exposure time in the Epelle at al. (2022) [29] study was shorter, but the ozone concentration was higher and the disinfection results obtained were much better than those obtained by us. This confirms that a higher ozone concentration is recommended for a shorter ozone time. In our studies in series 1-5, after 20 minutes of ozonation, we obtained a low efficiency of fungal elimination (0.0-33.5%) (Table 1, Figs. 2-6). In most cases, this efficiency increased 30 minutes after the end of the process (from 13% to 100.0%). In a study by Epelle et al. (2022) [29], despite the higher concentration of ozone (2 ppm), the elimination of fungi was also not sufficient. The highest disinfection efficiency was obtained after 16 minutes of exposure and decrease was 53% in the case of C. albicans yeast strain and only 35% for the *A. fumigatus*. These results show that removing fungi from the air requires longer exposure to ozone than bacteria.

Compared to series 1-2 and series 3 (Table 1; Fig. 4), the approximately tenfold higher VFR value (0.036 m3/s) resulted in

a decrease in the average ozone concentration to 0.293 ppm, which resulted in a lower disinfection effect in the case of mesophilic and psychrophilic bacteria. The same trends were observed in series 4 and 6 (Table 1; Fig.5 and 7). However, it was noted that a VFR value of 0.036 m^3/s in series 3 still allowed to maintain a higher ozone concentration after finishing the ozone generation for additional 2 minutes) and the exposure time of microorganisms was extended, resulting in greater disinfection than in series 4 and 6. Higher VFR values resulted in an immediate decrease in ozone concentrations after ozone generation was completed.

Surprising results of disinfection efficiency were obtained in the case of fungi. For the tested real conditions in which the research was conducted, it is difficult to find any trend regarding the dependence of fungal elimination on ozone concentration and VFR values. One would expect that in the series with the highest ozone concentrations, this efficiency would also be the highest. This has not been confirmed for either the 1 or 2 series. In the case of 5 series (simultaneous use of 2 ozone generators), the average ozone concentration was the highest (0.96 ppm), but due to the high VFR value (0.122 m^3/s), this concentration increased linearly to reach the maximum concentration point (1.4 ppm) after 20 minutes of ozonation, and after the generators were stopped, it dropped drastically (after 5 min to 0.4 ppm). In this series, immediately after ozonation, the reduction in the number of fungi was 33.5%, and after 30 minutes from the end of the ozonation process, it was already a reduction of 100%. For comparison, in the case of the 6th series, where the average ozone concentration was more than 4 times lower (0.23 ppm) than in the 5th series, immediately after ozonation the reduction in the number of fungi was 88.0%, and after 30 minutes from the end of ozonation it was only 59.0%. Therefore, it is difficult to identify any tendencies and mechanisms here.

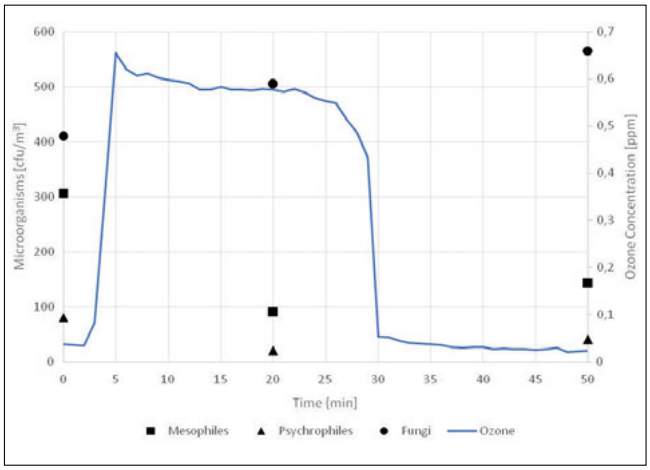

Figure 3.
Changes in the concentrations of ozone and airborne microorganisms occur before, immediately and 30 minutes after the ozone treatment is completed. The VFR of air is 0.004 m^3/s (series 2)
Rysunek 3. Zmiany stężenia ozonu i liczebności mikroorganizmów w powietrzu przed, bezpośrednio po i 30 minut po zakończeniu ozonowania. Strumień objętości powietrza równy 0,004 m^3/s (Seria 2)

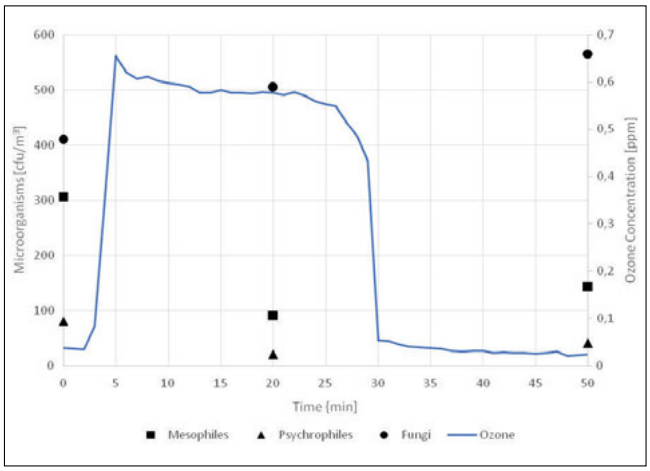

Figure 4.
Changes in the concentrations of ozone and airborne microorganisms occur before, immediately and 30 minutes after the ozone treatment is completed. The VFR of air is 0.036 m^3/s (series 3)
Rysunek 4. Zmiany stężenia ozonu i liczebności mikroorganizmów w powietrzu przed, bezpośrednio po i 30 minut po zakończeniu ozonowania. Strumień objętości powietrza równy 0,036 m^3/s (Seria 3)

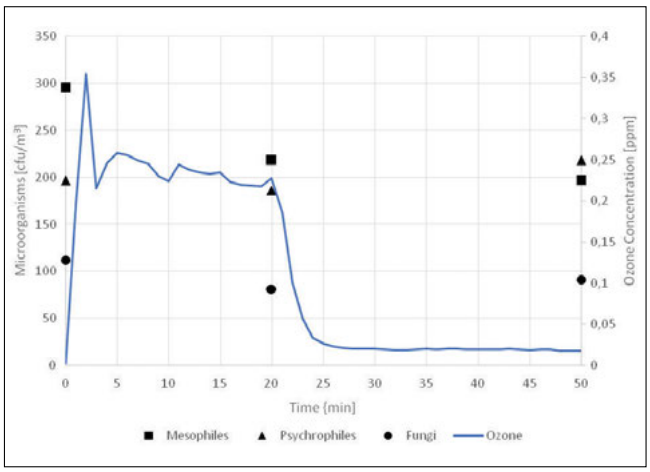

Figure 5.
Changes in the concentrations of ozone and airborne microorganisms occur before, immediately and 30 minutes after the ozone treatment is completed. The VFR of air is 0.109 m^3/s (series 4)
Rysunek 5. Zmiany stężenia ozonu i liczebności mikroorganizmów w powietrzu przed, bezpośrednio po i 30 minut po zakończeniu ozonowania. Strumień objętości powietrza równy 0,109 m^3/s (Seria 4)

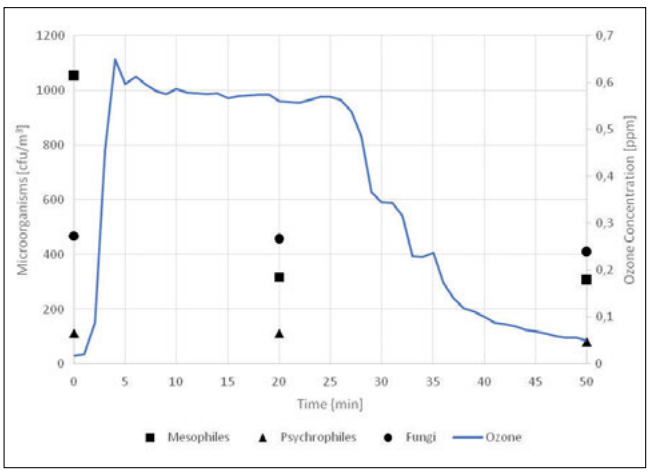

Figure 2.
Changes in the concentrations of ozone and airborne microorganisms occur before, immediately and 30 minutes after the ozone treatment is completed. The VFR of air is 0.004 m^3/s (series 1)
Rysunek 2. Zmiany stężenia ozonu i liczebności mikroorganizmów w powietrzu przed, bezpośrednio po i 30 minut po zakończeniu ozonowania. Strumień objętości powietrza równy 0,004 m^3/s

Relative humidity and temperature

The survival of microorganisms after ozonation depends, among others on environmental conditions, including temperature and RH [2]. The presented studies were carried out in real conditions, related to the variability of these factors, which made it difficult to unambiguously estimate their impact on the effectiveness of disinfection. It is reported that the increase in temperature increases the rate of ozone decomposition and decrease the effectiveness of

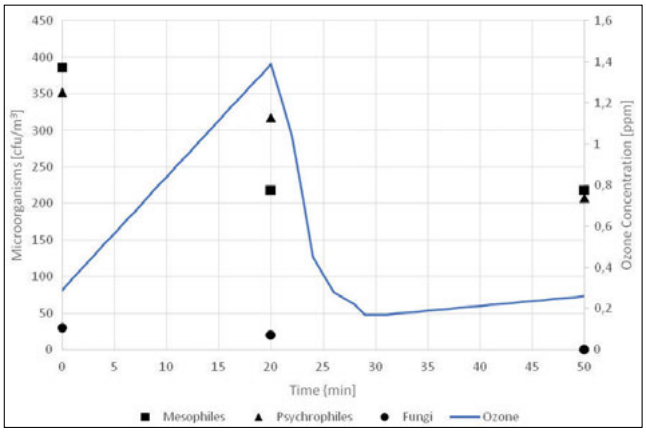

Figure 6.
Changes in concentrations of ozone and airborne microorganisms before, immediately and 30 minutes after the end of ozone treatment. The VFR of air is 0.122 m^3/s (series 5). During this series, two ozone generators were used simultaneously to produce ozone with a capacity of about 2000 g/min
Rysunek 6. Zmiany stężenia ozonu i liczebności mikroorganizmów w powietrzu przed, bezpośrednio po i 30 minut po zakończeniu ozonowania. Strumień objętości powietrza równy 0,122 m^3/s (Seria 5). Podczas tej serii, dwa generatory ozonu używane były jednocześnie do produkcji ozonu w ilości około 2000 g/min

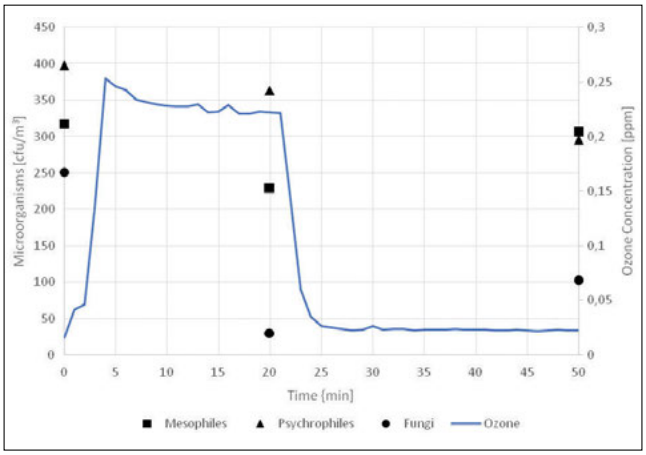

Figure 7.
Changes in the concentrations of ozone and airborne microorganisms occur before, immediately and 30 minutes after the ozone treatment is completed. The VFR of air is 0.136 m^3/s (series 6)
Rysunek 7. Zmiany stężenia ozonu i liczebności mikroorganizmów w powietrzu przed, bezpośrednio po i 30 minut po zakończeniu ozonowania. Strumień objętości powietrza równy 0,136 m^3/s (Seria 6)

Table 1. Percentage reduction of airborne microorganisms after the ozonation process under real conditions (A – immediately after the ozonation process; B – 30 min after the ozonation process is completed)
Tabela 1. Procentowa redukcja mikroorganizmów unoszących się w powietrzu po procesie ozonowania w warunkach rzeczywistych (A – bezpośrednio po procesie ozonowania; B – 30 min po zakończeniu procesu ozonowania)

							Reduction level [%]:		
Series	Volumetric ventilation air flow rate s[m^3/s]	Average ozone concentration over 20 min of ozonation [ppm]	RH Input/Output [%]	Inlet/Output Temperature [°C]			Bacteria mesophilic	Psychrophilic bacteria	Mold fungi
1.	0,004 (±0,003)	0,572 (±0,031)	43.2/59.0	21.1/15.5		A	69.8%	10.0%	2.5%
						B	71.0%	28.0%	13.0%
2.	0,004 (±0,003)	0,585 (±0,007)	44.0/59.4	20.5/15.0		A	70.2%	75.3%	0%
						B	53.0%	50.0%	0%
3.	0,036 (±0,018)	0,293 (±0,062)	55.5/69.3	20.4/13.3		A	31.7%	32.7%	27.8%
						B	26.0%	-0%	41.0%
4.	0,109 (±0,027)	0,241 (±0,052)	56.9/68.8	18.9/7.0		A	26.1%	5.5%	27.7%
						B	0%	0%	18.0%
5.	0,122 (±0,031)	0,96 (±0,041)	58.3/72.1	17.2/-3.0		A	43.5%	9.7%	33.5%
						B	43.0%	41.0%	100.0%
6.	0,136 (±0,034)	0,23 (±0,028)	60.2/78.0	18.0/2.2		A	27.9%	8.7%	88.0%
						B	4.0%	26.0%	59.0%

disinfection [2]. In the case of a high RH value, the ozonation process leads to the formation of free radicals, which increase the effectiveness of disinfection. In the study of Neves et al., 2023 [30], the interior of a city bus was exposed to ozone. With the same ozone concentration and exposure time, there was a more than 99.9% reduction in the number of microorganisms when the relative humidity was >90%, and a reduction in microorganisms of <90% for a lower relative humidity. Studies by Mazur-Panasiuk et al. (2021) [31] have shown that ozonation at low relative humidity (21.8%) can be considered ineffective even after 120 minutes and an ozone concentration of 7.3 ppm. In dry conditions, exposure of microorganisms to ozone does not lead to the desired disinfection effect. Ozone is most efficient in the presence of water or in high humidity conditions. Mazur-Panasiuk et al. (2021) [31] found that the results of ozonation at a relative humidity level of 50-70% were highly effective (reduction of microorganisms by 99.99%) due to the more intensive production of highly reactive hydroxyl radicals. In dry air, the disinfection procedure required a much longer exposure time. Such an effect was not observed in our studies if only the effect of relative humidity on disinfection efficiency was considered. An RH value below 50% was recorded in series 1 and 2, where the highest efficiency of elimination of both groups of bacteria was obtained, while in series 3-6 the relative humidity values were above 50% (55.5% to 60.2%), but the reduction of the number of bacteria was less effective. On the other hand, the dependence of disinfection effectiveness on RH was observed in the case of fungi. In series 1-2 with RH values below 50% (43.2% and 44.0%, respectively), the fungal reduction ranged from 0.0% to 13.0%, while in series 3-6 with RH values above 50% (from 55.5% to 60.2%), the fungal reduction ranged from 18.0% to 100%. No effect of temperature was observed.

Low temperatures lead to an increase in the bactericidal properties of ozone in high humidity conditions, as its molecular stability increases, while high temperatures lead to an increase in ozone reactivity. It was observed that in the range of temperatures and relative humidity values for which the study was conducted, the VFR value of

the air had a greater impact on the concentration of ozone in the air than the above-mentioned factors [4], [7] .

Conclusions

The effectiveness of disinfection in the case of mesophilic and psychrophilic bacteria was dependent on ozone concentration and exposure time. The higher ozone concentration and the longer exposure time found in some series increased the effectiveness of the disinfection. At a constant rate of ozone production, the ozone concentration decreased markedly as the volumetric flow rate (VFR) of the air increased. This, in turn, led to a decrease in the effectiveness of disinfection. Taking into account the influence of relative humidity and temperature, a relatively high temperature (22°C) combined with an RH value of about 50% allowed to obtain the highest disinfection efficiency against mesophilic and psychrophilic bacteria. In the case of fungi, humidity above 50% contributed to an increase in the efficiency of ozonation. Real-time measurements of ozone concentrations during the ozone generation process indicate that ozone concentration are generally relatively constant. The obtained results indicate that conducting air disinfection in real conditions in order to achieve proper effectiveness requires appropriate configuration of parameters such as VFR, ozone concentration, exposure time. The influence of RH and temperature should also be taken into account. In further research, we plan to use hybrid systems to remove the indoor airborne bacteria and fungi that will combine different sterilization methods.

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